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Stratigraphic Distribution of Amino Acids in Peats from Cedar Creek Bog, Minnesota, and Dismal Swamp, Virginia[1]

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ABSTRACT

The concentration of several alpha-amino acids obtained by acid hydrolysis at various levels in Cedar Creek Bog and Dismal Swamp has been determined. Seven amino acids are consistently present in the natural accumulations: glycine, aspartic acid, glutamic acid, threonine, alanine, valine, and leucine. Alanine occurs in the highest concentrations, attaining about 22 μmol/g in the samples investigated. Free amino acids were absent or very rare in both the bog waters and waters squeezed from the peats. Attempts to detect proteins in these substances by means of paper electrophoresis were unsuccessful. Fibrous proteins may be present, however.

In both peat deposits there is a greater concentration of amino acids at depths of a few feet than in the uppermost peat layers. Hydrolysis of a humic acid preparation from Minnesota peat yielded the same suite of amino acids given above. It is therefore suggested that the amino acids in our peat hydrolyzates mainly occurred adsorbed by, or linked to, the humic acid micelles rather than as proteinaceous compounds.

The distribution of amino acids in Cedar Creek Bog peat suggests two periods of trophication, one beginning approximately 12,000 years ago in Mankato time and ending with the so-called thermal maximum perhaps as late as 6,000 years ago, the other beginning after the thermal maximum and continuing to the present. Evidence of more than one cycle of peat development in the Lake Drummond area of Dismal Swamp is lacking.

INTRODUCTION

The nitrogen content of peat deposits reaches 4% or more but probably averages less than 1% (Waksman 1938, p. 277). In Cedar Creek Bog, nitrogen content is 2.3% at 4–5 feet, 2.8% at 7–8 feet, 3.4% at 9–10 feet, and less than 1% at most deeper levels (Swain and Prokopovich 1954, p. 1186). The nitrogen generally is considered to occur in the form of proteinaceous compounds that have formed as cell constituents of microorganisms. The latter have grown in the subsurface portions of the peat bog from the decomposition products of the accumulating higher plants (Waksman 1938, p. 115). The downward increase in total nitrogen, typically found in low-moor peats (Waksman and Stevens 1928) is offered in support of this concept. The information resulting from the present study suggests, on the other hand, that the nitrogenous materials in the two lowmoor bogs we studied are associated primarily with humic acids rather than with proteins in the strict sense, although existence of peptide linkages in the material cannot be ruled out.

The vertical distribution of amino acids in the upper 2–4 meters of lake sediments in southern Ontario was studied by Kleerekoper (1957, p. 124). He found that the amino acids showed little change with depth

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in the sediments of some of the lakes, an increase in depth in others, and a decrease in still others. The changes apparently are related to variations in the sand, clay, and perhaps marl content of the lake sediments, rather than to migrations within the sediments as here suggested for Cedar Creek Bog and Dismal Swamp peats. Kleerekoper was successful in isolating a rather large number of amino acids by sulfuric acid hydrolysis from the lake sediments, including alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, hydroxyproline, leucine and isoleucine, lysine, methionine, proline, serine, tourine, threonine, and valine. An excess of nitrogen not accounted for by the amino acids is thought to occur in lignin-nitrogen complexes, such as humic acids.

The distribution of humic substances in several European lakes was thought by Züllig (1956, p. 109–111) to result not only

TABLE 1. *Vertical distribution of amino acids in hydrolyzates of Cedar Creek peat, Minnesota*

Depth ft	Type of sample	Bog Zones	Wt. of wet sample (g)	Moisture %	Amino acids (parts per 10,000)									
					Glycine	Asp. acid	Glut. acid	Threonine	Alanine	Valine	Leucine	Σ	C%	N%
2–3	Copropelic peat	II_f	17	89	1.7	0.94	0.60	0.29	2.94	1.12	2.06	9.65	47.23†	2.31
5–6	Peaty copropel	β_p	21	90	3.53	1.95	5.68	2.83	21.92	2.83	9.33	48.07	47.59	2.77
8–9	Sapropelic copropel	β_c	20	87	0.90	0.60	3.55	1.77	11.25	5.82	7.82	31.71	48.01	3.36
11–12	Sapropelic copropel	β_c	21.5	68	1.74	1.16	3.49	1.74	19.77	3.02	7.37	38.29	9.78	0.90
14–15	Copropelic marl	M_c	23	61	0.43	0.28	1.58	0.80	0.49	1.67	1.48	6.73	15.13	0.75
17–18	Copropelic marl	M_c	29	60	0.42	0.28	1.37	0.67	—	0.15	0.15	3.04	—	—
20–21	Copropelic marl	M_c	26	60	0.01	0.07	0.03	0.02	—	—	—	0.13	—	—
23–24	Copropelic marl	M_c	23	60	0.05	0.03	0.48	0.24	—	0.14	0.20	1.14	—	—
26–27	Dark copropel	β_c	12.5	79	0.28	0.19	—	—	0.27	0.17	0.40	1.31	—	—
29–30	Copropelic marl	M_c	30	60	0.02	0.01	0.25	0.14	—	0.25	0.45	1.12	—	—
32–33	Copropelic marl	M	37	60	0.04	0.03	0.02	0.10	0.03	0.02	0.01	0.25	—	—
35–36	Sideritic marl	M_{sid}	24	70	0.07	0.05	0.80	0.39	0.63	0.19	—	2.13	8.47	0.9
37–38	Sideritic sand	Γ_s	42.9	60	0.007	0.005	0.24	0.12	1.66	0.21	0.31	2.55	—	—
41–42	Sand	Γ_s	28	35	—	—	—	—	—	—	—	—	—	—
Bison rib	Dark, humus impregnated		10 (dry)		0.236* 0.224*h*	2.996 *ly*	0.254	0.030	0.098*a* 0.0864*pa*	0.198	0.288	4.410	—	—

* probably includes serine and aspartic acid

† C and N determinations were made previously on cores collected near "b" on Figure 1. Depths are, respectively: 4–5, 7–8, 9–10, 11–12, 12–14, and 35–36 feet.

ly = lysine *a* = alanine *pa* = phenylalanine *h* = histidine

TABLE 2. *Vertical distribution of amino acids (in parts per 10,000) in acid hydrolyzates of Dismal Swamp peat, Virginia*

Depth ft	Type of sample	Bog Zones	Wt. of wet* sample (g)	Glycine	Lysine	Glutamic acid	Threonine	Alanine	Valine	Leucine	Histidine	Σ
1–2	Reddish brown woody peat	II_0	28	0.32	0.08	0.16	0.08	2.49	0.99	0.59	—	4.71
2–3	Reddish brown woody peat	II_f	27	—	—	—	—	.005	.005	.005	—	.015
3–4	Reddish brown woody peat	β_f	40	1.46	0.37	7.16	3.54	11.81	2.16	1.51	0.50	28.52
4–5	Dark brown copropelic fibrous peat	β_c	60	0.44	0.11	0.54	0.27	2.88	0.59	0.95	0.82	6.60
5–6	Dark brown copropelic fibrous peat	β_c	45	0.53	0.13	0.91	0.45	4.91	0.86	1.74	0.18	9.71
6–7	Sandy, dark fibrous copropel	β_c	45	0.14	0.04	0.37	0.18	1.43	0.37	0.66	0.11	3.30

* Moisture content approximately 90%.

from autochthonous production but from allochthonous introduction of organic material. An increase of organic nitrogen 5–10 cm beneath the surface in the bottom sediments of eutrophic Zürichsee was attributed to allogenic material. The present writers recognize this possibility but believe that because trophication has been generally progressive in Cedar Creek Bog and Dismal Swamp, the humic substances and associated amino acids, whether of authigenic or allogenic nature, should typically increase upward to the surface. That this is not the case is believed to have resulted from downward concentration of soluble humic material rather than from increased allogenic influx of humus at some time in the past.

METHODS

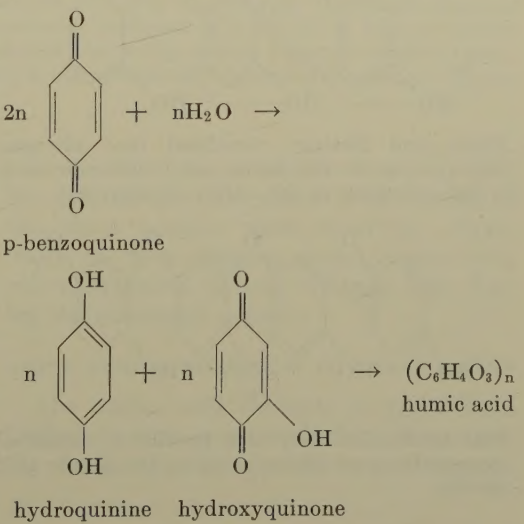
Wet samples of the peat and underlying marl were hydrolyzed with 6N hydrochloric acid for 22 hours under reflux. The hydrolyzates were reduced to dryness and taken up in 5 ml of isopropyl alcohol. Approximately 1 microliter of the concentrates of each sample was placed on sheets of Whatman No. 1 filter paper together with a mixture of known amino acids; solvent, butanol:acetic acid:water (40:10:50); chromatograms stained by dipping in ninhydrin in acetone. The developed chromatograms were then scanned with a photometer, and the concentrations in the wet sediment were determined as shown in Tables 1 and 2, using the area x density method (Block *et al.* 1955, p. 72). The approximate dry weight values can be determined from the moisture content. If an average weight of a mole of the amino acids studied here be taken as 100 g, the values in Tables 1 and 2 can be read in μmol/g. Two-dimensional chromatograms were run to verify the identity of the individual amino acids (Fig. 2B). A composite chromatogram of the suite of Cedar Creek Bog samples is shown in Figure 2A.

AMINO ACID DISTRIBUTION IN CEDAR CREEK BOG

The vegetational zones and collecting localities at Cedar Creek Bog are shown in Figure 1. In the upper five feet of the bog there is a downward increase in all the amino acids from as little as 2-fold in aspartic acid to as much as 10-fold in alanine. The upper 4–5 feet of the sediments of Cedar Creek Bog are characterized by a predominance of forest and *Sphagnum* peat; below lies sedge peat and copropel (gyttja). Part of the downward increase in amino acids is believed to be due to the originally greater protein content in the sedge peat and copropel than in the forest peat. Additional downward concentration of amino acids may be accomplished by means of aqueous solutions of "humic acid" to which the amino acids are attached. Because the copropelic material is relatively impervious, the bog waters from above probably do not circulate across these layers to any extent. The amino acids brought into the upper part of the copropel by the moving bog waters probably are concentrated and held there.

Water humus (Waksman 1938, p. 290) is soluble or occurs as gel suspensions in the waters of bogs and especially of dystrophic lakes. A special study of some of this material was made by Shapiro (1957), who found these "humolimnic" acids to be of phenolic or enolic nature with an empirical formula of $(C_{50}H_{62}O_{27}N)$. He believed the nitrogen to be an impurity. The present writers suggest that amino acids may be attached to these humic materials perhaps by means of an amine bridge as postulated by Thiele and Kettner (1953)[2] and thus subject

[2] These authors proposed that humic acid formed from quinones, such as p-benzoquinone probably by bacterial decomposition:



to transportation vertically or laterally within the bog.

Samples of the surface bog water and of water squeezed from the peat samples were concentrated and tested for proteins by means of paper electrophoresis, and for amino acids by paper chromatography. Water soluble proteins apparently are absent, and free amino acids are lacking or nearly so in these waters. When the peat samples were extracted with cold dilute HCl, differences in amino acid content of the extracts differed from that of the hydrolyzate only in total amount. The data suggest that free amino acids are rare in Cedar Creek Bog. The amino acids freed by decomposition of the bog vegetation are either: (1) immediately utilized by other bog organisms, or (2) are bound to the humic acid benzenoid structures that are forming at the same time.

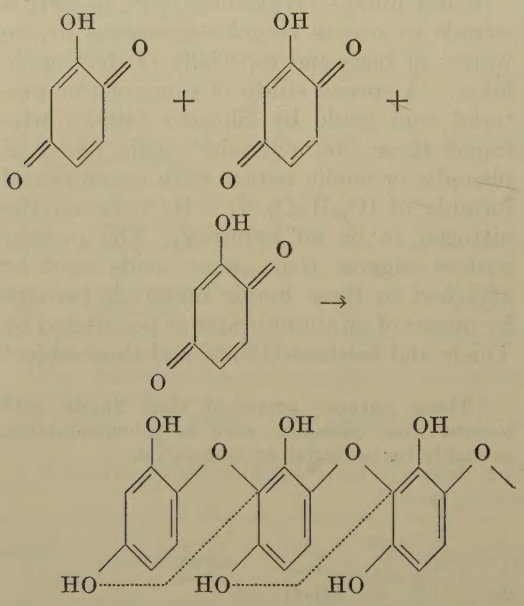
Supporting evidence for this conclusion is the presence of amino acids in, or associated with, humic acid preparations as noted by several observers (Okuda and Hori 1954, 1956; Hayashi 1956; Bremner 1955; Blumentals and Swain 1956). At the present time it is not known whether the amino acids are simply adsorbed to the humic micelle or linked to it by —CH_2—, —N(H)—, —CO—, —S—, or other group. The near neutrality of the bog waters would favor the transportation of the humic acid micelles and their associated amino acids laterally and downward, thus helping to account for the observed distribution in the upper 10–12 feet. The downward moving humic acids would be precipitated in part by flocculation on contact with the underlying marl, as well as by the imperviousness of the copropel.

Beneath the copropel there is a sharp decrease in amino acid content of the underlying marl that results not only from the lower total organic content of the marl, but also from the lack of downward migration of the high molecular-weight substance through the copropel. There is a general downward decrease in the individual amino acids between depths of 14 and 26 feet; in fact alanine is absent at several levels. This is very likely the result of downward concentration in the relatively porous marl.

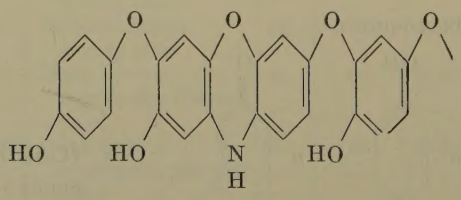
The increase of several of the amino acids in the dark copropel layer at 26–27 feet may be the result both of original high protein composition and of downward concentration. This impervious layer probably impedes further concentration downward of amino acids from the overlying marl. Beneath the dark copropel, the generally low amino acid content is a reflection both of low total organic content and downward concentration. Alanine, particularly, seems to move downward to the nearest impervious or flocculating layer; in this case sideritic marl and sand.

To test stability of the Cedar Creek

Footnote 2 continued.
The humic acid benzenoid system may form by polymerizing the hydroxyquinone, as follows:



Thiele and Kettner visualized that nitrogen might occur in the humic acid molecule as a bridge substance of the nature of oxazine:



This mechanism may also provide a means of incorporation of amino acids in the humic acid micelle.

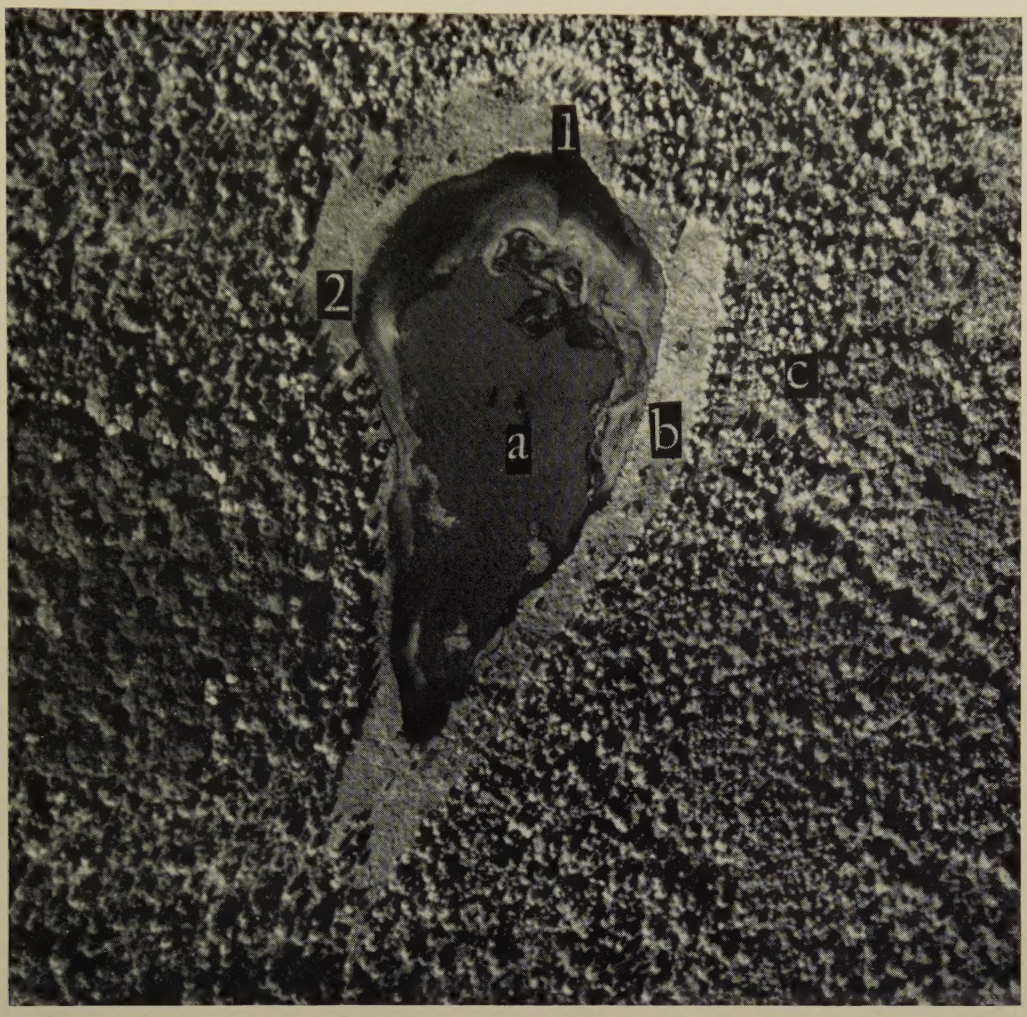


FIG. 1. The pond and surrounding part of Cedar Creek Forest, Anoka County, Minn. The larger end of the pond (a) is toward the north and the pond is 725 feet long. The forest contains an extensive growth of white cedar (*Thuja*) and mat of *Sphagnum* moss (c). The sedge border of the pond contains *Decadon* (swamp loosetrife), *Typha* (cattail) and scattered tamarack (*Larix*) (b); a band of denser tamarack adjoins the sedge mat. The growth of pondweed beds in the pond is shown by shades of gray. Locality 1, cores obtained for amino acid study; locality 2, cores obtained for radiocarbon study. Photograph by Walter J. Breckenridge, August, 1953.

amino acids, two fresh samples of modern marly copropel from the Cedar Creek pond were placed in bottles on top of a nuclear generator and left for 6 months during the spring and summer of 1956. The purpose of this experiment was to determine whether the X-rays and gamma rays from the generator together with bacterial action inside the glass bottles would affect the amino acid content. Extracts of these samples were cochromatogrammed with a sample that had been stored at room temperature (Fig. 2C). No differences in the amino acid content of the three samples were observed. Physically, all three samples, stored in their own water, appeared to have changed little during the six-month period.

AMINO ACID DISTRIBUTION IN DISMAL SWAMP

The amino acid contents of peats from Dismal Swamp were studied to determine: (1) whether the apparent downward concen-

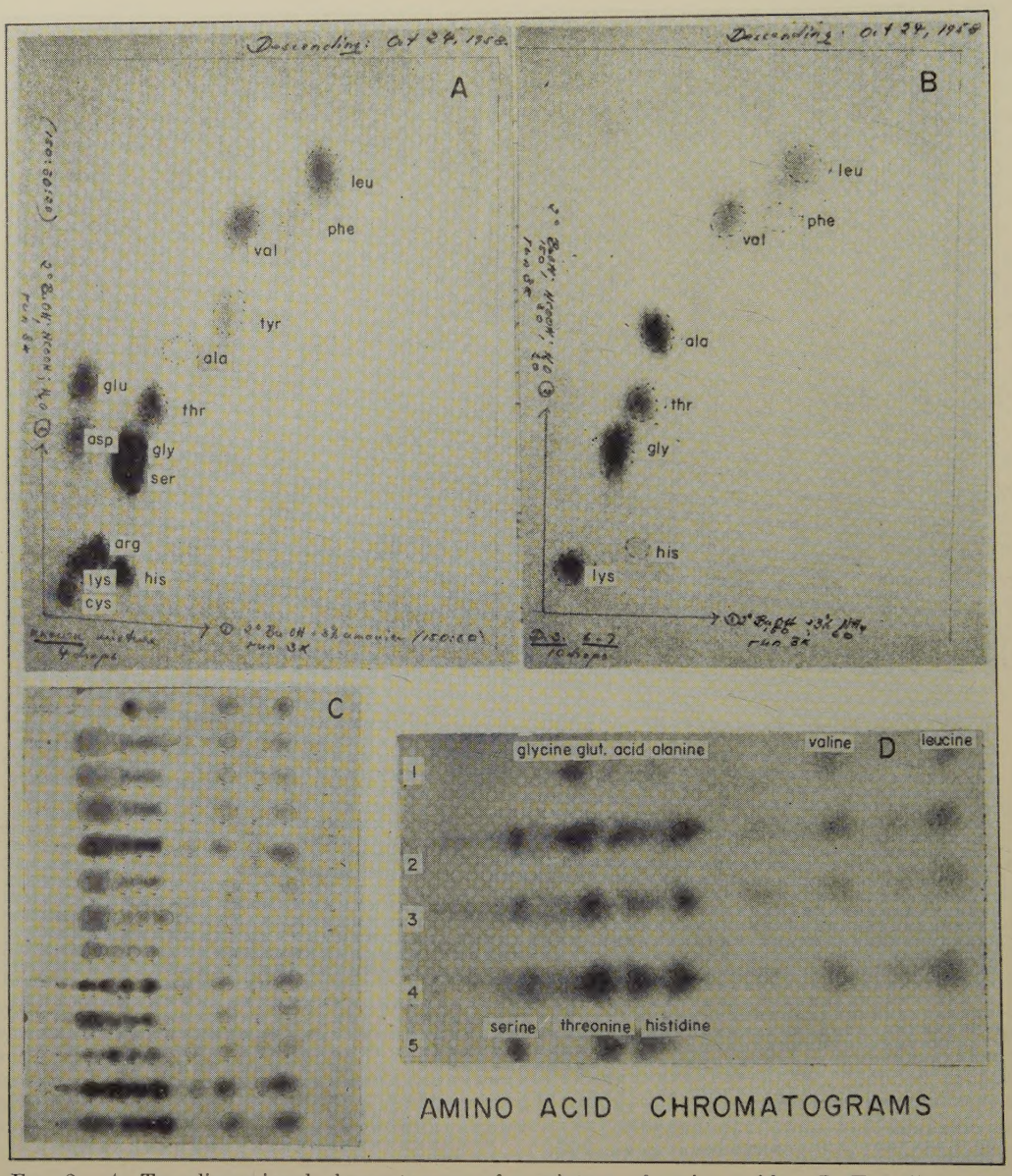


FIG. 2. *A*. Two-dimensional chromatogram of a mixture of amino acids. *B*. Two-dimensional chromatogram of 6–7 foot Dismal Swamp hydrolyzate. *C*. Composite one-dimensional chromatogram of hydrochloric acid hydrolyzates from Cedar Creek Bog, locality 1; origin of each chromatogram is on the left; depths in feet reading upward are: 2–3, 5–6, 8–9, 11–12, 14–15, 17–18, 20–21, 23–24, 26–27, 29–30, 32–33, 35–36; top chromatogram represents a mixture of glycine + glutamic acid (darkest spot), alanine, valine, and leucine; large spots near base (on left hand side) of each chromatogram from 20 to 36 feet are due to iron compounds. *D*. Composite one-dimensional chromatogram of two samples (2, 3) of marly copropel from Cedar Creek Bog irradiated for 6 months with gamma rays and X-rays, compared to a sample (4) kept at room conditions for the same period, showing similarity of all three samples; 1 and 5 are standards.

tration noted in Cedar Creek Bog occurs in Dismal Swamp as well, and (2) whether the amounts and kinds of amino acids showed similar relations to stratigraphy in the two areas.

The samples of Dismal Swamp peat were obtained on Lake Drummond about 500 feet north of the mouth of Dismal Swamp Canal feeder. The locality is close to Osbon's (1919, p. 48) locality D where he found 10 feet of peat having, at a depth of 4 feet, the following moisture-free composition: C, 20.66%; volatile matter, 53.02%; ash, 26.32%; N, 1.84%; S, 0.31%. This is referred to as "black gum" peat because of the abundance of the gum tree *Nyssa biflora* in the area. The following are also found in the black gum association (Osbon 1919, p. 43): *Fraxinus caroliniana* (water ash), *Berchemia scandens* (rattan), *Gelsemium sempervirens* (yellow jessamine), *Bigonia capreolata* (cross vine), *Taxodium distichum* (bald cypress), *Acer rubrum* (red maple), and *Nyssa aquatica* (cotton gum).

At the locality sampled there were about 7 feet of peat, the upper 4 feet consisting of reddish brown, porous, punky, woody peat. The lower 3 feet are dark brown, copropelic, fibrous peat, very sandy in the lower 6 inches and rest upon sand. The peat waters are more acidic than those of Cedar Creek Bog ranging between 4.5 and 5.

The amino acids attain their highest concentration in the lower part of the porous woody peat. In the hydrolyzate from 2–3 feet beneath the surface no amino acids were detected. Because of the rapid growth of vegetation in this swamp, the surface layers probably contain undecomposed protein or polypeptides plus humus. The amino acids may occur in both types of compounds here. The humic acids and associated amino acids appear to undergo concentration downward to the base of the woody peat where they are precipitated. The absence of amino acids in the 2–3 foot layer is believed to be a result of the downward movement of the water-soluble humic acids that are continually leached from the upper layers of peat as fast as they form. The effect on amino acid distribution of the many swamp fires that have swept the area is not definitely known; perhaps there has been merely a general loss of organic constituents rather than of particular constituents. It does not seem reasonable that reduction of amino acids in the 2–3 foot layer resulted from their destruction by heat, because the peat of this layer is not observably different from that above or below.

The age of the sands underlying the peat may be Pliocene (Shaler 1890, p. 315) or Pleistocene (Osbon 1919, p. 50), but whatever their age, there is no evidence in the samples studied to suggest more than one cycle of peat development in the Lake Drummond area of Dismal swamp. The lower dark layers represent lake peat that was succeeded by the reddish brown forest peat. In other parts of Dismal Swamp thicker peat deposits have been reported, and in those places the bog development may have been more complex.

PROFILE DEVELOPMENT IN THE PEAT BOGS

The distribution of the layers in the typical peat bog from the viewpoint of genesis and distribution of decomposition products can be compared to a forest or meadow soil profile. The symbols suggested here are for use in describing the several peat layers. The active decomposition layer (II_0) can be likened to the A_0 soil zone of decomposition; the underlying leached zone (II) in the peat is comparable to the A_1–A_2 soil zone; and the β zone of concentration of humic acids is similar to the B soil zone. The zones in the peat bog of which Cedar Creek Bog, Minnesota, is a type example are summarized as follows:

II_0 zone; Fermentation layer, characterized by moderate content of carbohydrates, lignin, bitumens, humic substances and amino acids; late eutrophic and/or dystrophic stage of bog.

II_f, II_s, etc. zone; Bleached layer in forest peat, sedge peat, etc., indicated by subscript; characterized by lower content of carbohydrates and amino acid, and higher content of lignin and humic substances; eutrophic and/or dystrophic stage of bog.

β_p, β_c, etc. zone; Accumulation layer in peat, copropel, etc., shown by subscript; characterized by high content of humic substances and bitumen, increased quantities

of amino acids associated with humic materials, reduced lignin, and markedly reduced carbohydrates, eutrophic and/or dystrophic stage of bog.

M zone; marl layers deposited during photosynthesis of phytoplankton, representing interval of introduction of calcium bicarbonate brought into the lake in solution from surrounding uplands; the marl is frequently copropelic and with siderite layer at base; early eutrophic stage of bog.

Γ_s, Γ_c, etc. zone: sub-bog material consisting of sand, clay, etc.

POSSIBLE EFFECTS ON C^{14} DETERMINATIONS

The effects of downward concentration of amino acids on radiocarbon age determinations would be to increase the apparent age of the leached zone and to decrease that of the accumulation layer. The quantities of amino acids in Cedar Creek Bog are such that the effects of downward migration would be of the order of .01 to 5%; *i.e.*, in the richest layer, 5–6 feet, if all the amino acids at this level had been carried from above, an error of about 2% of the total sample would be involved. The carbon content of the sample is approximately 45% (Swain and Prokopovich 1954, p. 1186).

In addition, downward concentration of some other organic materials such as the humic acids also may involve errors of the order of 5% or more, judging from preliminary data we have assembled on the distribution of the humic materials. The total movement of organic material may involve quantities of 1 to 10% of the sample, thereby introducing corresponding errors in the radiocarbon determinations. Objects such as bone, shell, or wood that tend to adsorb migrating substances may be particularly prone to error in radiocarbon data. Removal of the humic material from the specimen prior to treatment for radiocarbon dating might help to avoid these possible errors.

LATE PLEISTOCENE EVENTS RECORDED IN CEDAR CREEK BOG

The succession of amino acids and of marl and peat in Cedar Creek Bog suggest two periods of lake development. The first stage began approximately 12,000 years ago (Wright and Rubin 1956) with the deposition of the basal sideritic marl in a relatively cold, but post-glacial, climate, the latter indicated by the pine pollen of Zone B (Flint and Deevey 1951, p. 272). An approximate correlation of the base of Cedar Creek Bog with the Two Creeks horizon of north-central U. S. and the Alleröd horizon of Europe is suggested by C^{14} dates (Flint 1957, p. 395). After about 12 feet or more of marl had accumulated, the trophication of the lake had progressed to the stage in which dark copropel (gyttja) formed plentifully on the lake floor, at which time the vegetation surrounding the lake was dominated by oak (pollen zone C-2) (Flint & Deevey 1951, p. 272), suggesting that the post-glacial warm period had been reached. A radiocarbon date of 7988 ± 420 years from the top of pollen zone B was obtained (Flint & Deevey 1951, p. 272) from the marl beneath the dark copropel.

The apparent original distribution as well as subsequent concentration in the humus of the amino acids in Cedar Creek peat and marl suggest two periods of trophication. The thermal maximum in the Cedar Creek area may have been of short duration, a few hundred years or less, because only copropel, not sedge or forest peat, is found. Whether or not aridity was necessary to reinstate the deposition of marl that overlies the dark copropel is uncertain. Elevated temperature and lessening of precipitation probably resulted in general lowering of the groundwater level, and of the zone of weathering during the thermal maximum. Lowering of temperature and rise of groundwater level in the time subsequent to the post-glacial warm interval has resulted in calcium carbonate again being leached from the glacial drift to be carried into Cedar Creek Bog. This second cycle of trophication has apparently continued until the present time with no pronounced interruptions.

CONCLUSION

Additional work should be done on the fulvic and humic acid fractions of peat, as well as on the alkali-insoluble humin to determine the nature of the nitrogenous com-

pounds and the precise way the amino acids appear to be associated with them. Amino acids have been found in rocks as old as Eocene (Vallentyne 1957) and in fossils as old as Devonian (Abelson 1954, 1957), wherein association with humic substances is likely. Various other bituminous rock samples as old as Precambrian have been investigated for amino acids by the writers with variable success. The mechanism by which certain amino acids are preserved in some rock materials and not in others should be studied.

The widespread distribution of a few amino acids in bog and lake deposits is of interest because several of the so-called essential amino acids are absent from the list or occur only infrequently (isoleucine, phenylalanine, methionine, arginine, histidine), and because several that are found in oil shale and in fossil shells also are absent. The medium in which the amino acids occur may be significant, because *Bison* rib from a Minneapolis peat bog contained several amino acids (histidine, lysine, serine (?), and phenylalanine) not detected or rare in the peat.

The amino acids observed by Abelson (1957, p. 279) to occur most commonly in fossil *Mercenaria* from Pleistocene, Pliocene, and Miocene strata are glutamic acid, glycine, alanine, isoleucine or leucine, proline, and valine. Of these only proline was not commonly found in the peat deposits. Aspartic acid which Abelson suggests is relatively easily hydrolyzed to ammonia and malic acid at moderately elevated temperatures is commonly present in the peats and may serve as a geologic thermometer in interpreting the past history of organic sediments.

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Bacterial Populations in Sea Water as Determined by Different Methods of Enumeration[1]

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ABSTRACT

Five different cultural and two direct microscopic methods were employed for estimating the abundance of bacteria in samples of sea water collected from both oceanic and neritic areas. The cultural methods included macrocolony counts on nutrient agar, silica gel, and membrane filters, microcolony counts on membrane filters, and the extinction dilution method. Direct microscopic counts were made of microbes on membrane filters and of microbes transferred from membrane filters to glass slides. Direct counts showed the presence of from 13 to 9,700 times as many bacteria as cultural methods. The extinction dilution method and the microcolony membrane filter method gave counts 20 and 35 times higher, respectively, than did any of the macrocolony methods. Direct microscopic counts on membrane filters were approximately 150 times higher than plate counts, and the numbers of microbes transferred from membrane filters to glass slides were approximately 2,000 times higher than plate counts. In all of the cultural methods, a peptone-yeast extract medium was used.

Differences in the abundance of microorganisms obtained by the various methods are attributed to a variety of factors: the presence of bacteria in aggregates, selective effects of the media, and the presence of inactive cells. A marked decrease in bacterial numbers was observed just below the thermocline as reflected in the macrocolony methods but not by the direct microscopic methods. A considerable population of spirilli-like forms was noticed directly under the microscope but not after cultivation.

INTRODUCTION

Studies of microorganisms in natural waters involve enumeration as a general index of activity and as a measurement of biomass. Little has been done in evaluating the intrinsic significance of the results of counting methods. Although cultural methods of enumeration are widely used, they yield only a small percentage of the microorganisms actually present (ZoBell 1946). The application of direct microscopic methods in water bacteriology led to counts 200 to 5,000 times higher than plate counts (Butkevich 1932, 1938). Radsimovsky (1930) explained this difference in bacterial numbers by the presence of autotrophic and organophobic organisms. After comparing the bacterial numbers and the oxygen demand of corresponding water samples, Butkevich and Butkevich (1936) concluded that a considerable portion of the bacteria must be present in the resting stage. However, Alfimov (1954) doubted the occurrence of many "dead" bacteria in sea water and sediments. Thus, there exists uncertainty concerning the state of bacteria in natural waters as well as the value of the various methods used for enumeration.

In the present investigation, the abundance of microorganisms in oceanic and neritic areas of the Pacific Ocean was investigated by seven methods of enumeration.

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METHODS

Sampling locations

Stations where sea water samples were collected:

1) On October 31, 1957, sea water was ob-

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tained from an area approximately equidistant between Baja California and Guadalupe Island (29° 18′ N latitude; 116° 56′ W longitude) at depths of surface, 25, 50, 75, 100, and 200 meters.

2) On December 20, 1957, sea water was obtained from an area 16 miles off the coast of San Diego, California (32° 38′ N latitude; 117° 32′ W longitude), at depths of surface, 25, 50, 75, 100, and 200 meters.
3) On the following dates, surface sea water samples were collected off the Scripps Institution of Oceanography pier: January 3, 1958; January 17, 1958; and February 6, 1958 (a local tide pool was also sampled on this date).

Types of water samplers used:

1) Water samples obtained at sea for cultural methods of enumeration were collected in sterile J-Z bacteriological water samplers using collapsible rubber bottles (ZoBell 1946).
2) Water samples collected for direct methods were obtained in Van Dorn (1956) samplers.
3) Surface water samples obtained near shore were collected in sterile one-liter glass-stoppered bottles.

Media

Basal medium, 2216E (Oppenheimer and ZoBell 1952)—Bacto-peptone, 5 g; Bacto-yeast extract, 1 g; $FePO_4$, 0.01 g; Bacto-Agar used for pour plate method, 15 g; aged sea water (salinity adjusted to 28 g/L), 1,000 ml; pH adjusted to 7.6 to 7.8. Medium 2216, not containing yeast extract, was found superior to other media for development of maximum numbers of aerobic heterotrophic marine bacteria (ZoBell 1941). This medium was improved by the addition of yeast extract as confirmed by Carlucci and Pramer (1957). Two other media were also used in one experiment.

Succinate medium—Succinic acid, 2.0 g; NH_4NO_3, 1.0 g; K_2HPO_4, 0.1 g; aged sea water (salinity adjusted to 28 g/L), 1000.0 ml; pH adjusted with NaOH to 7.6 to 7.8.

Casein hydrolysate medium—Enzymatic casein hydrolysate (NBC), 1.0 g; NH_4NO_3, 1.0 g; K_2HPO_4, 0.1 g; aged sea water (salinity adjusted to 28 g/L), 1000.0 ml; pH adjusted to 7.6 to 7.8.

Methods of enumeration

Agar pour plate method—Samples of sea water estimated to produce between 20 and 500 colonies per plate were transferred aseptically to sterile (90-mm) plastic petri plates. In certain oceanic areas samples as large as 5 to 10 ml may be required. When working at sea the plates were supported by a weighted swinging table.

Sterile nutrient agar was transported in screw-cap prescription bottles and remelted at sea. Plates were poured with nutrient agar cooled to 40–45° C and mixed thoroughly with the sample. After solidifying on the swinging table, the plates were incubated for at least five days before counting colonies.

Extinction dilution method—Medium 2216E broth was prepared and sterilized in 9 ml amounts in test tubes. One ml portions of the water samples were inoculated into five replicates of the broth. Each of the replicates was diluted by 10-fold increments from 10^0 to 10^{-6} ml. Growth was determined by turbidity in the broth after incubation and the most probable number (MPN) of microorganisms estimated from the distribution of positive and negative tubes from the table of Hoskins (1934).

Silica gel method—The advantages of silica gel as a solidifying agent for microbiological media and some practical improvements in its preparation have been presented by Pramer (1957). In this investigation the method was modified for use with sea water.

The resin column and silica sol were prepared as described by Pramer (1957). The silicate source was $Na_2SiO_3 \cdot 9H_2O$ (3 % SiO_2, w/v). The silica sol was sterilized by autoclaving at 10 lbs pressure for five minutes.

Three parts of the sterile silica sol were mixed with one part of sterile double-strength 2216E broth. Full strength aged sea water was employed as the liquid phase of the nutrient solution. This gave a final concentration of sea water of 25 % (salinity of 8.5 g/L). Gelling time with this mixture

was approximately six minutes. However, when triple strength artificial sea water (Lyman and Fleming 1940) was substituted for full strength aged sea water, the gel formed almost instantly. Careful adjustment of the pH to 7.5 to 7.8 with NaOH resulted in a firm silica gel.

Macrocolony membrane filter method—From 2 to 50 ml of sea water sample were passed through a sterile 47-mm type PH Millipore filter to concentrate the microorganisms. The filters were sterilized at 112°C for a few minutes and stored in sterile distilled water until used. The inoculated filters were placed on adsorbent cellulose pads soaked with 2216E nutrient solution in 60-mm petri plates. Three such plates were incubated in a 150-mm petri plate which served as a moist chamber. The time and temperature of incubation for each water sample are presented in tables of data. Macrocolonies are defined as those which are large enough to be visible to the naked eye. The filters were stained with Löffler's methylene blue in order to make very small colonies visible.

Microcolony membrane filter method—Employing methods described by Jannasch (1958), colonies developing on nutrient-impregnated membrane filters were counted under a microscope at a magnification of 430 to 970×. In the microcolony method a 25-mm Millipore filter was used to concentrate microbes in water samples, and only 1 per cent as much nutrient was added as in the macrocolony method. Decreased incubation times and low nutrient concentration avoid the possible toxicity of the latter and regulate overgrowth of rapidly growing cells.

Direct microscopic method on membrane filters—Water samples were treated with formaldehyde, and several dilutions of each sample were filtered to obtain optimal distribution of microorganisms on the filter surface (Beling 1950, Alfimov 1954, and Jannasch 1953, 1958). Sterile distilled water was added during the last stages of filtration to remove salts (Jerusalimsky 1932). Staining with methylene blue yielded better results in counting microcolonies, but a 1 per cent solution of erythrosine in 5 per cent phenol often proved superior for differentiating individual cells. Even with this staining technique, the low optical contrast of the microscopic fields occasionally made it difficult or impossible to differentiate small microbial forms from inorganic particles on the filter surface. Indistinct forms were not counted.

Mechanical cleaning of surfaces which come in contact with the water sample is required in direct methods rather than sterilization. Consequently, carefully cleaned and dried Van Dorn (1956) samplers were used for collecting large volumes of sea water for this direct method. The number of bacterial cells remaining on the inner surface of the samplers was estimated by examining the water used to rinse the samplers by the Cholodny method. The contamination was less than 0.1 per cent of the total cell count.

Cholodny method—The Cholodny (1928) method involves microscopic enumeration of microorganisms on glass slides after concentration by filtration. The Cholodny method was modified somewhat in the following experiments. Depending on the expected bacterial density, 100 to 2,000 ml of the sea water sample obtained with the Van Dorn (1956) sampler were fixed immediately with 1 per cent formaldehyde. The sample was passed through a membrane filter using the apparatus illustrated in Figure 1. By turning the two-way stopcock (E), the vacuum filtration was stopped when two to three ml were left above the filter. Salts were removed by washing several times with sterile distilled water. Care was taken to leave a few ml of solution above the filter. Two precautions in the design of the apparatus prevented deposits from sticking to the filter surface which would be difficult to remove quantitatively: (1) an ultrafine sinter-glass plate (B) with a lower filtration speed than the membrane filter and (2) a collodium loop (C) 2 mm wide, which was rotated mechanically on the filter surface during filtration (Bachman 1926). The numbers of microorganisms remaining on the membrane filters were determined by microscopic examination and served as a control. The bacterial cells remaining amounted to 1.5 per cent on the average and did not exceed 8.5 per cent of the total count. The concentrated sample was transferred quantita-

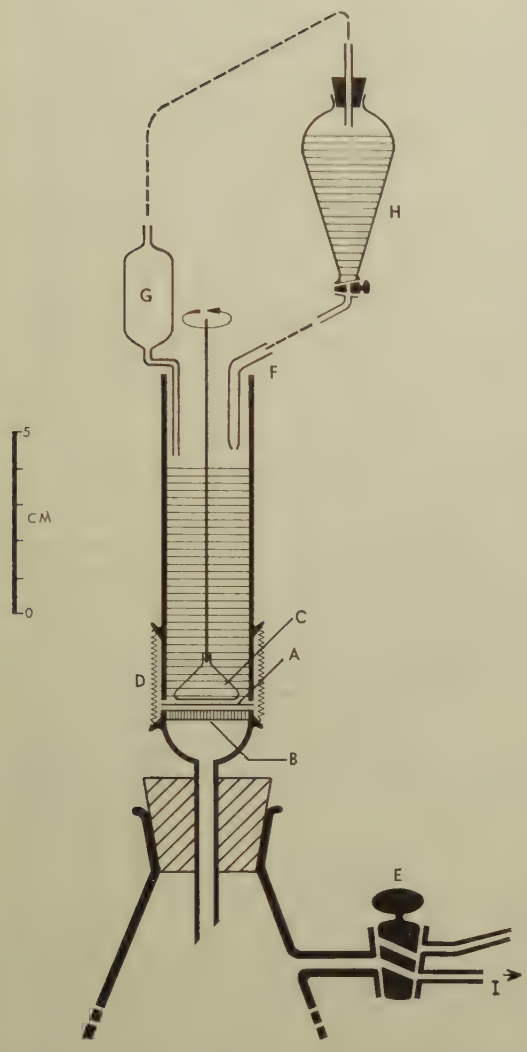


FIG. 1. Filtration apparatus for the Cholodny method. (A) membrane filter, (B) ultra-fine sinter glass plate, (C) stirring loop of collodium, (D) spring, (E) two-way stopcock, (F) sample entry, (G) level control, (H) separatory funnel (reduced in scale) containing sample, and (I) outlet connected to vacuum.

tively into small test tubes and adjusted to a certain volume (3 to 5 ml) with 5 per cent formaldehyde. Formaldehyde also was used to rinse the filter surface. With a calibrated capillary tube or micropipette, 0.01 ml of the concentrated sample was transferred to a slide, dried, and stained with a 2 per cent solution of erythrosine in 5 per cent phenol for at least five hours. The size of the dried area was measured with an ocular grid at an enlargement of 35 times. Possible contamination during 15 to 29 minutes of filtration was checked by examining filtered water. Bacterial cells introduced from the air did not influence the cell count. The per cent error of the counts obtained by this method is based on examination of 25 microscopic fields for each sample.

In the tables, per cent error is calculated by $S\bar{x}/\bar{x} \cdot 100$ where $\bar{x}$ is the mean and $S\bar{x}$ is the standard deviation of the mean.

RESULTS

The numbers of microorganisms detected by the various methods of enumeration from an oceanic area (Station 1) are presented in Table 1. The water temperatures at the various depths were recorded by a bathythermograph. The abundance of bacteria recovered from an area approaching the neritic zone (Station 2) are plotted in Figure 2. The number of bacteria recorded from the neritic waters off the Scripps Institution of Oceanography pier (Station 3) are presented in Table 2.

Evaluation of methods

As observed in Tables 1 and 2 as well as in Figure 2, the agreement among bacterial counts obtained by the agar pour plate method, silica gel method, and the macrocolonies on membrane filters was quite close. Submerged growth in the agar pour plate method which might tend to favor the development of anaerobic and microaerophilic microorganisms was no higher than that obtained by macrocolonies on membrane filters. In addition, no significant deviation was observed in samples from different depths of the sea. These results are not in agreement with those of Carlucci and Pramer (1957) who found as many as 30 to 40% more colonies arising after pouring plates than on surface-inoculated plates. These investigators have suggested the existence of a large percentage of microaerophilic and anaerobic bacteria in Atlantic ocean water.

ZoBell and Conn (1940) have shown that some marine bacteria are thermosensitive and are killed at the congealing temperature of agar. However, there is no direct evidence that the heat of the agar adversely affects the microorganisms developing on

TABLE 1. *Comparative numbers of microorganisms per ml indicated by different methods at Station 1*
Per cent error in parentheses. In all cultural methods the incubation temperature was 18 ± 1°C.

Depth m	Water temp. °C.	Plate[1] method		Serial dilution method (MPN)		Macrocolonies[1] on MF 5 Days	Microcolonies on MF 3 Days	Direct counts on MF
		5 Days	21 Days	5 Days	21 Days			
Surface	20.1	6 (10%)	11 (10%)	7	7	8 (9%)	68 (3.1%)	244 (1.8%)
25	20.1	14 (12%)	14 (12%)	5	5	14 (7%)	31 (5.4%)	262 (2.1%)
50	19.0	9 (19%)	10 (6%)	7	7	10 (12%)	30 (8.7%)	166 (6.4%)
75	15.0	1	2	0	0	6 (14%)	24 (11.2%)	147 (3.1%)
100	13.0	4 (14%)	6 (29%)	2	2	1 (15%)	29 (6.6%)	82 (11.3%)
200	10.0	3 (39%)	4 (58%)	2	2	5 (12%)	46 (4.0%)	179 (7.2%)

[1] An average of three replicates.

TABLE 2. *Comparative numbers of microorganisms per ml indicated by different methods of enumeration at Station 3*
Per cent error in parentheses. In all cultural methods the incubation temperature was 18 ± 1°C.

Area sampled	Water temp. °C.	Plate method		Silica gel method		Serial dilution method (MPN)		Macroc. on MF[4] 5 days	Microc. on MF 3 days	Direct count on MF	Cholodny method
		5 days	20 days	5 days	20 days	5 days	20 days				
Off pier	16.2	218[1] (8%)	239 (8%)	191[1] (7%)	218 (17%)	3500	3500	191 (11.2%)	1344 (6.2%)	1619 (7.4%)	25,800 (0.7%)
Off pier	16.0	37[2] (10%)	56 (11%)	17[1] (32%)	43 (14%)	220	350	61 (15%)	822 (9%)	1810 (5.3%)	41,100 (1.2%)
Off pier	15.9	25[3] (12%)	71 (5%)	—	—	64	1800	19 (13%)	108 (5.8%)	2290 (4.4%)	55,400 (3.2%)
Tide pool	16.2	75[3] (10%)	107 (9%)	—	—	700	9500	68 (17%)	136 (4.8%)	1780 (6.2%)	46,100 (2.2%)

[1] An average of 10 replicates.
[2] An average of 6 replicates.
[3] An average of 4 replicates.
[4] An average of 5 replicates.

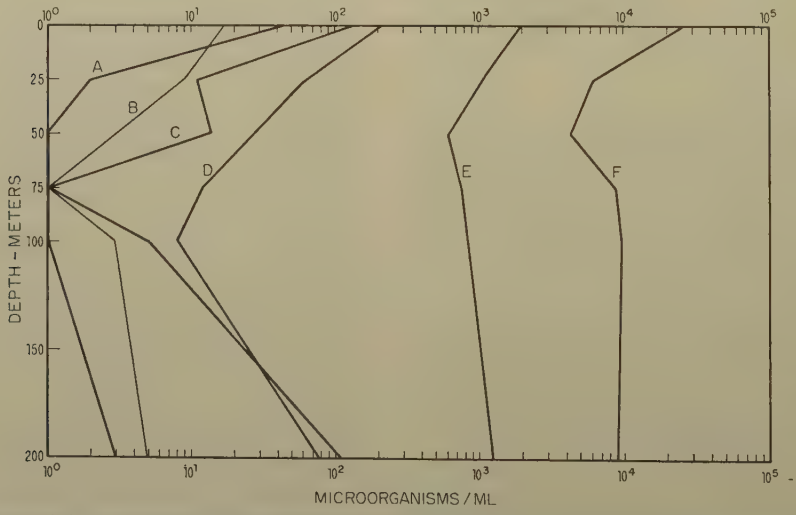


FIG. 2. Bacterial populations at depths obtained by different methods from Station 2. (A) agar pour plate method, (B) macrocolonies on membrane filters, (C) extinction dilution method, (D) microcolonies on membrane filters, (E) direct count on membrane filters, and (F) Cholodny method.

TABLE 3. *Number of microcolonies per ml as influenced by nutrients, period of incubation, and concentration of the water samples from Station 1*

Medium	Peptone-yeast extract (diluted 1:100)									Succinate (diluted 1:100)									Casein hydrolysate (diluted 1:100)								
Amount of water filtered...	2 ml			4 ml			8 ml			2 ml			4 ml			8 ml			2 ml			4 ml			8 ml		
Period of incubation (18 ± 1° C) hr	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
Depth, meters																											
Surface	22	59	**68**	29	52	—	34	—	—	11	14	17	9	17	**31**	16	29	22*	22	25	34	19	41	—	16	**53**	—
25	20	23	31	28	**55**	—	48	54	—	12	14	20	16	17	**26**	19	21	24	21	24	36	16	28	31	24	**39**	—
50	14	25	30	16	36	**52**	21	44	—	0	4	7	4	13	14	7	10	**18**	0	11	29	8	19	28	6	23	**44**
75	6	23	24	8	37	**46**	10	36	—	0	3	9	4	5	11	6	**19**	10*	0	0	14	1	6	9	6	8	**15**
100	0	13	29	6	21	30	6	27	**36**	0	8	14	0	12	18	13	17	**22**	0	4	13	0	16	21	5	**26**	20*
200	15	27	**46**	22	39	—	23	38	—	8	14	22	2	10	**26**	4	16	19	8	12	31	6	18	26	13	**42**	—

— = overgrowth of membrane filters

* = decrease in the number of colonies due to overgrowth

Bold face type = maximum number of microcolonies per depth and medium used

TABLE 4. *Ratio of microbial counts, range of values, and per cent error of five different methods compared to plate counts computed from all data*

	Plate method	Macro-colonies on MF	Serial dilution method	Micro-colonies on MF	Direct counts on MF	Cholodny method
Mean	1	1.16	21.8	32.1	147	2100
Range	—	0.2–4.0	0.3–35	1.6–34	13–840	108–9700
Per cent error	—	17.1	19.2	12.7	8.5	20.7

agar plates as compared to those on membrane filters or silica gel.

Bacterial counts from agar pour plates were higher than those from silica gel plates (Table 2). This was true at sea as well as in near-shore samples. This effect may be attributed to specific salt requirements of marine bacteria (ZoBell 1941). In addition, the composition of unwashed agar is variable (Yaphe 1957), and micronutrients in the agar might be expected to stimulate marine bacteria (MacLeod *et al.* 1954, Jones 1957). ZoBell (1941) found about three to four per cent of the bacteria in the sea digest agar.

The most probable number (MPN) of microorganisms determined by the extinction dilution method was considerably higher than the macroscopic colony count, although the nutrient solution used in this method was identical with that used in the other cultural methods (Fig. 2 and Tables 2 and 4). This condition was not evident in oceanic water samples (Table 1). The increased counts in the majority of samples due to the liquid medium are attributed to the dispersion of bacterial aggregates and clumps on detritus as regulated by the surface tension depression of the medium. La Riviere (1955) has demonstrated that 1% peptone drops the surface tension of water by 18 dynes/cm, and 1% yeast extract by 25 dynes/cm. Surface tension depressions approaching this order of magnitude would be sufficient to disrupt bacterial aggregates causing the observed increase in the final count (Jones and Jannasch 1959). These results agree with those of Butkevich (1932) who obtained from ten to a hundred times as many bacteria by extinction dilution as by plate counts, using water samples from the Barents Sea. Butterfield (1933) reported good agreement between plate counts and the MPN of extinction dilution employing 50 replicates for the plates and 50 of each of three dilutions using *Escherichia coli*. He found 37% higher values by extinction dilution using the same experimental design when *Aerobacter aerogenes* was used as the test organism. The difference between the bacterial numbers obtained using the two test bacteria was ascribed to the greater tendency for the *Aerobacter aerogenes* cells to clump due to the presence of mucoid substances.

In the microcolony technique on membrane filters three small petri dishes were

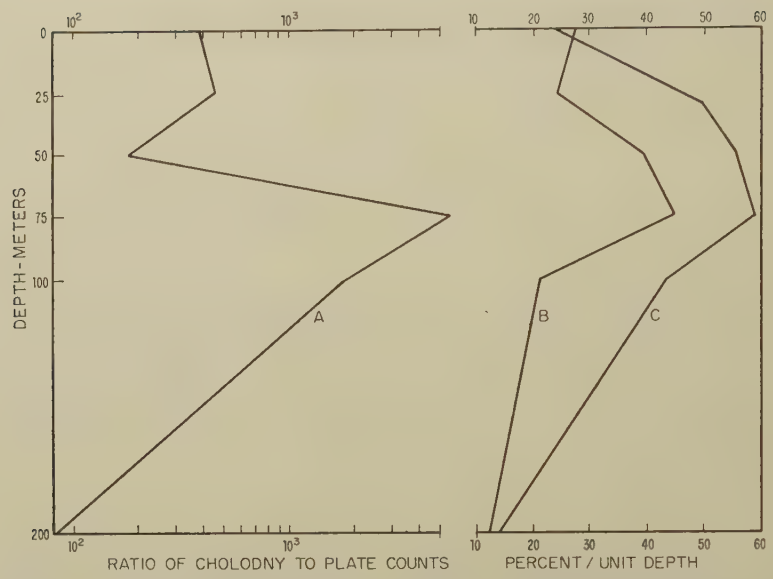


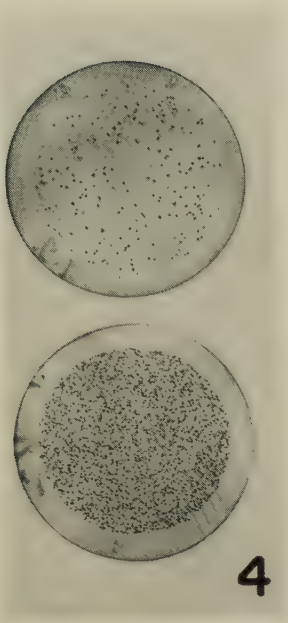
FIG. 3. Variations with the vertical distribution of microbial populations at Station 2. (A) ratio of microorganisms/ml obtained by the Cholodny method to microorganisms/ml recorded by agar pour plate method, (B) per cent of spirilli-like organisms per unit depth as determinated from the Cholodny count, and (C) per cent of aggregated microorganisms per unit depth as determined by Cholodny method.

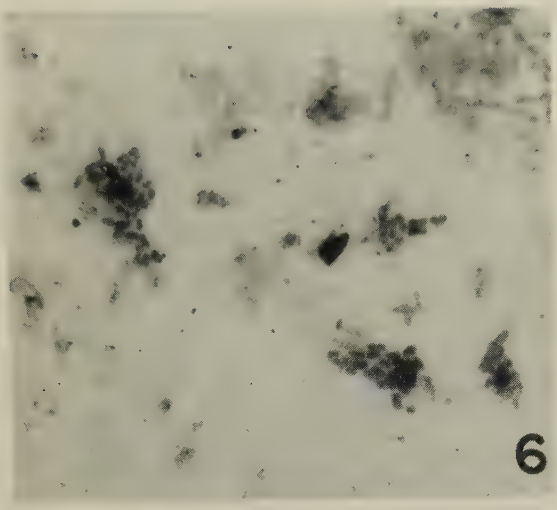
placed in a larger one which served as a moisture chamber during incubation. Each of the small petri dishes contained three membrane filters which were divided, again, in three parts before incubation. In this way, three combinations of three different factors (nutrients, time of incubation, and concentration of sample) were compared, and 27 different values were obtained for each of the samples tested (Table 3).

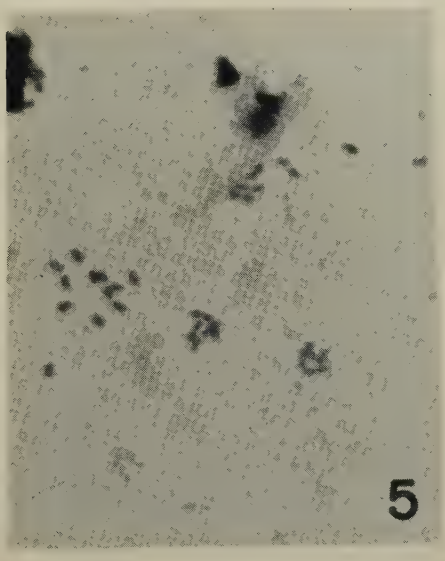
The bacterial numbers obtained by the microcolony method demonstrate dependence on the incubation time. After 24 hours, groups of 2 to 20 cells developed on the membrane filter surface (Figs. 5 and 6). Single cells, presumably inactive, were not counted. Often colonies appear to have spread (Fig. 5). After approximately 48 hours in these experiments, colonies were composed of several hundred cells (Figs. 7 and 8). In many cases, colonies had begun to merge in 72 hours. Therefore, the colony counts may decrease before the membrane filter has been overgrown completely (Table 3). Sometimes dense colony centers indicate the existence of several original loci (Fig. 9).

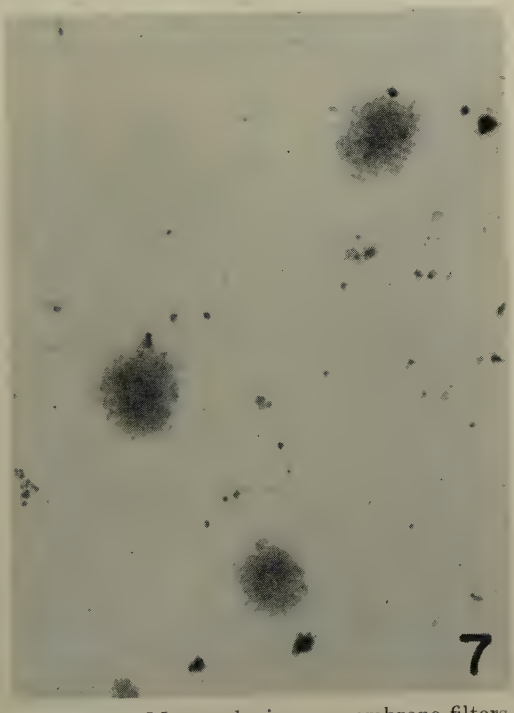
Deviations from this general pattern of development are caused by density of bacteria on the filter surface (Table 3). In general, maximum counts appeared earlier when larger amounts of sample were used. Due to the density of bacteria in the water sample the maximum count occurred at a characteristic place in the series of nine experiments. Only the counts in the same columns are comparable. Of the three media tested, the peptone-yeast extract medium proved superior as indicated by the development of more colonies in a short period of time with early overgrowth of the filters. Similar growth ratios for these nutrients were obtained by the agar pour plate method using the same water samples. Generally, higher counts obtained by this method may result from the low nutrient level. In only a few cases were macrocolonies apparent on these filters after several weeks of incubation. Comparing the photomicrographs of Frost (1921) taken from microcolonies on the surface of agar plates with the type of growth on membrane filters, no considerable difference was noticed (Fig. 10).

Direct microscopic examination of membrane filters yielded lower numbers than









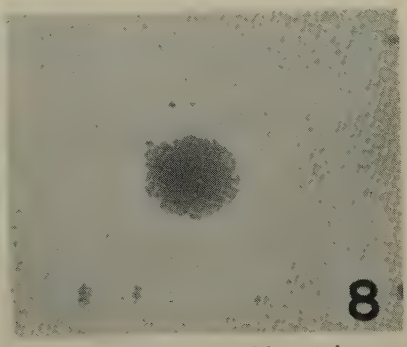
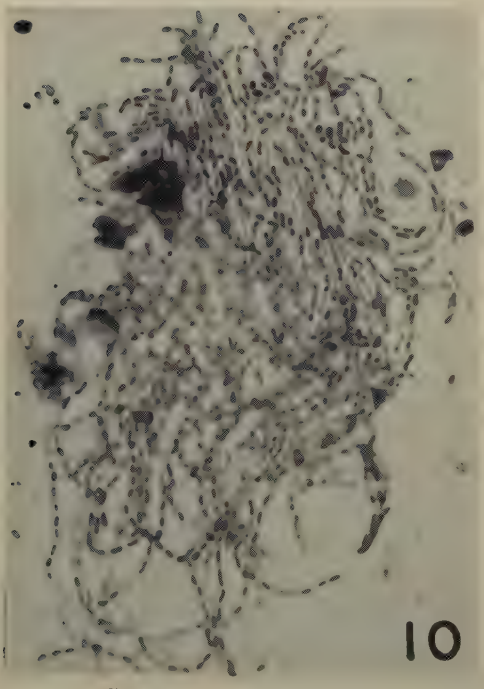


FIG. 4. Macrocolonies on membrane filters stained with Löffler's methylene blue stain.

FIGS. 5 and 6. Microcolonies on 2216E medium (diluted 1:100) after 24 hours incubation at 18 ± 1°C on membrane filters. Magnification 970×.

FIGS. 7 and 8. Colonies on 2216E medium (diluted 1:100) after 48 hours incubation at 18 ± 1°C on membrane filters. Magnification 500× and 700×, resp.



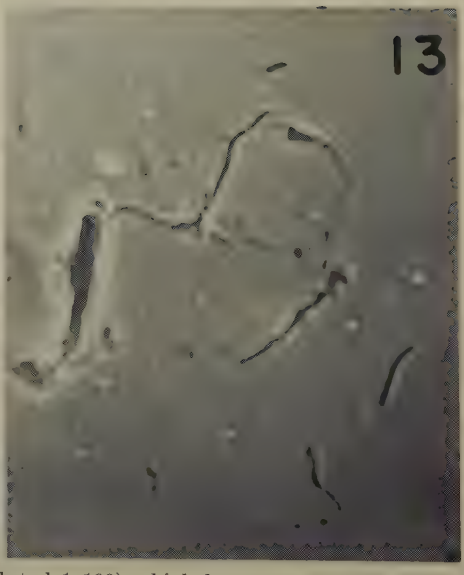


Fig. 9. Colonies on casein hydrolysate medium (diluted 1:100) which have grown together after 72-hours incubation at 18 ± 1°C on membrane filters. Magnification 150×.

Fig. 10. Typical colony of a chain-forming organism on membrane filter. Magnification 900×.

Figs. 11–13. Spirilli-like and filamentous microorganisms stained with erythrosine obtained by the Cholodny method from samples of 75 and 200 meters, respectively, at Station 2. Magnification 1500× Figures 12 and 13 reproduced from phase contrast.

those obtained by the Cholodny method (Table 4). Small and weakly stained cells may escape observation on the membrane filters due to the lower optical contrast of the preparation. The Cholodny method has been improved by the use of membrane filters of smaller porosity than those of Cholodny (1928, 1929) and Novobrantzev (1932), 0.3 μ as compared with 2.0 μ.

Evaluation of the vertical distribution of marine bacteria

As indicated in Table 1 and Figure 2, the results obtained by the various methods at depths down to 200 meters indicated a similar distribution of microorganisms in both vertical casts. The largest counts of bacteria occurred in surface waters, decreasing down to 75 meters, with an increase in abundance at 200 meters. Similar vertical distributions of microorganisms in the sea have been observed by Kriss and Rukina (1952), who demonstrated minimum bacterial counts at depths of 25 to 100 meters. This minimum corresponds roughly to the location of the thermocline.

A marked deviation of the ratio between direct microscopic counts and cultural method counts was observed at the various depths. The ratio of microorganisms obtained by the Cholodny method compared to the plate method at Station 2 are plotted at the various depths in Figure 3. These results indicate very few colonies developing on agar plates as opposed to relatively large numbers of cells observed directly, especially at 75 and 100 meters.

With both of the direct methods large spirilli-like organisms (4–15 μ long, 1–3 μ wide), as shown in Figures 11–13, were observed throughout the vertical casts. The long flagella (5–20 μ) were stained by erythrosine. Some of these organisms were motile in wet mounts prepared from water samples concentrated by filtration. The vertical distribution of these microorganisms in per cent of the Cholodny count is shown in Figure 3. None of these forms were observed in microscopic preparations made from colonies or dilution tubes, developing from the same water samples.

The percentage of microbes at various depths attached to detritus or observed in clumps as determined by the Cholodny method are plotted in Figure 3. The curve indicates less clumping where the plate count figures were highest (surface and 200 meters). In addition, the per cent errors of the direct counting methods (Table 1) show the lowest values in the same samples. The highest errors appear in samples of 75 and 100 meters.

DISCUSSION

Agar still appears to be the best solidifying agent for enumeration of bacteria at sea. However, silica gel has certain advantages for studying the nutritional requirements of bacteria, because it is a chemically defined substance and biologically inert. In addition, silica gel solidifies at any desired temperature within the biological range. Both of these solidifying agents are superior to gelatin, because many marine bacteria liquefy gelatin, which results in the merging of colonies before slow-growing bacteria have had time to develop visible colonies (ZoBell 1941).

Direct microscopic methods possess the advantages of revealing a more exact enumeration of the microorganisms in a sample than cultural methods regardless of their growth requirements or their physiological condition, and these results can be obtained in a very short period of time. However, there is no way to determine whether the bacteria observed are living or dead, and they cannot be used for cultural studies. Direct counts may be increased by the presence of non-proliferating, inactive, or dead cells. Karsinkin and Kusnetsov (1931) and Alfimov (1954), using erythrosine stain to differentiate living from dead protoplasm, found low percentages of dead bacteria in lake and sea water. Kusnetsov (1958) reported that dead bacteria constitute about 10 per cent of the total number of bacteria in lake water as determined by Peshkov's staining method. Strugger (1949), using acridine orange, obtained similar results in soil.

Direct microscopic methods are complicated by particulate matter in the sample simulating the appearance of microorgan-

isms. Clumps are difficult to count. Therefore, no organism was counted unless it could be distinguished clearly from detritus. Cocciform bacteria were counted only when clearly differentiated. Due to these precautions, the actual bacterial counts are probably higher than those reported for the direct microscopic methods. The staining reaction of erythrosine was not used to distinguish between organic and inorganic material or living and dead protoplasm as suggested by Karsinkin and Kusnetsov (1931).

Ratios comparing the results obtained by cultural and direct microscopic methods for all of our experiments are presented in Table 4. The range of comparable ratios in lake water determined by Kusnetsov and Karsinkin (1931) using an evaporation technique for concentrating the sample for microscopic examination was 1:2,000–4,000. Unfortunately, this method is not applicable for sea water samples. Collins and Kipling (1957) obtained from 6 to 11,000 times as many bacteria by their direct method as by plate counts from Lake Windemere North Basin water. Salimovskaya-Rodina (1938) found up to 5,000 times higher counts in lake water by direct microscopic methods than by plate counts. According to Butkevich (1938), these ratios increase with the decrease of organic matter in the water.

Bacterial populations were reported to be directly proportional to organic matter by Novobrantzev (1932) and Chartulari and Kusnetsov (1937). According to Kriss (1953), at depths descending from the thermocline the bacterial numbers are regulated by the concentration of organic matter. As suggested by Butkevich (1938), the the converse of curve A in Figure 3 may present a rough index of the relative distribution of organic matter with depth. In this respect, it is noteworthy that our highest numbers of microorganisms were at the surface in Pacific Ocean water as were those of Lloyd (1930) in the Clyde Sea. Another possible explanation for the deviation of the ratio between direct microscopic and cultural counts at various depths is the different nutritional requirements of the populations at these depths. The spirillilike organism is an example of a microorganism which escapes detection by cultural techniques. It is probable that other marine bacteria in these samples also did not develop into colonies with the nutrients used.

Another factor which decreases the plate counts is aggregation of bacteria as indicated by the direct methods. According to Jennison (1937) and Ziegler and Halverson (1935), the occurrence of clumps of bacteria is the main reason for the differences between direct microscopic and cultural counts of cells in cultures. A detailed study of the existence of microbial aggregates in the sea will appear shortly (Jones and Jannasch 1959).

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An Investigation of the Circulation over Second Point Bar, Lake Mendota

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ABSTRACT

The flow of water in the middle and surface layers around and over a lake bar was examined in terms of horizontal and vertical velocities, and divergence. These quantities were mapped and the patterns correlated with the general current velocity, wind conditions, and obstruction by the bar.

1) The bar caused two types of flow around it. In the unstratified layer above the thermocline the flow continued right over the bar, although the exact pattern of motion depended upon the current speed and the geography of the bar.

2) In the thermocline there was a strong tendency for the water to flow around the bar, although considerable upwelling and sinking of the layer occurred. Helical rotors appeared on either side of the bar.

3) Internally consistent and detailed patterns of circulation were observed on a small scale without excessive expenditure of time or money.

THE EXPERIMENT

During the summer of 1954 a detailed investigation of the water circulation around and over Second Point Bar, Lake Mendota (Fig. 1) was undertaken. The project was a joint effort of the University of Wisconsin Departments of Meteorology and Zoology, and was aimed primarily at the relationship between fish habits and circulation. This paper, however, will dèal only with the circulation patterns.

A network of stations marked by numbered buoys about 1000 feet apart was established over the area (Fig. 2). Three times a day for a number of selected days the temperature distribution and currents at one, two, five, and ten meters depth were measured at each buoy. From these data temperature and streamline charts were drawn. From the current observations both divergence and vertical velocities were derived. This was done by mapping as vectors all the current observations for each depth from a particular run. Then, by using Bellamy's (1949) method of computing divergence from a vector field, regions of convergence and divergence at one, two, five, and ten meters were determined and contoured. These values of divergence were next employed to calculate the vertical velocity at those same points. This was accomplished by means of the equation of continuity, which states that horizontal divergence or outflow from a volume of an incompressible medium must be compensated by vertical inflow and vice versa. Thus, starting from an assumed vertical velocity of zero at the water's surface, and adding successively the vertical velocity increment due to the divergence in each layer, the vertical velocity of the water was obtained and mapped at each observational level. Finally, by combining the observed horizontal velocity components with the computed vertical velocity components, a three-dimensional vector field was constructed. Through this vector field streamlines were drawn to give a picture of the actual flow around and over the bar.

We wish to thank particularly Miss Alison Lathbury and Mr. William Bunge, Jr. who helped collect and process much of the data, and Mr. David Griggs who gave us many hours of his own time on the drafting.

RESULTS

Rather than describe the individual results of the whole series of cruises, the following discussion will deal with a typical situation to exemplify the overall pattern that emerged from the study.

During the 17–19 hour cruise of July 20,

1954, the wind was from the west, and there was a general eastward set of the current in the Second Point area. Figure 3 presents the observed current flow at one, two, five and ten meters. As the current passed over the bar at one and two meters, it was forced into a standing wave of considerable amplitude. The configuration of standing waves in the surface layer seen in Figure 3, can be shown, by reference to the whole set of data, to have been largely dependent upon the surface current speed: lower speeds produced standing waves of shorter length but of greater amplitude. As will be pointed out subsequently, the position of these standing waves was associated with a definite pattern of divergence in the surface layer. At five and ten meters the flow was largely deflected by the bar; most of the water at this depth appears to have been eventually forced northward so that it flowed around the bar rather than over it.

As the streamlines on the diagrams are spaced in inverse proportion to the speed of the current, disappearance of streamlines downstream can be interpreted as horizontal convergence, which in turn must mean vertical divergence (by the equation of continuity). Conversely, appearance of streamlines downstream indicates horizontal divergence, which similarly means vertical convergence.

The values of divergence computed by the Bellamy method for the 17–19 hour cruise of July 20, 1954 are mapped at each depth in Figure 4. The positive values indicate divergence, and negative values represent convergence. The units ($\times 10^{-4}$) are changes in area per second; thus, 1.0 means the area increased (or diverged) 1.0×10^{-4} of its initial size in a second, while -0.5 means the area decreased (or converged) 0.5×10^{-4} of its initial size in one second.

Figure 5 shows the vertical velocities for the same cruise derived by integrating the divergences from the surface downward. Positive values indicate upwelling, and negative values indicate sinking water. In the surface layers the locations of the various areas of rising and sinking water were found to depend partially upon the

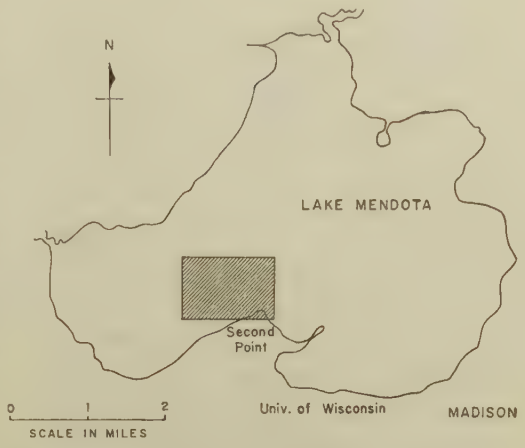

FIG. 1. Lake Mendota, Madison, Wisconsin. Hatching indicates area of investigation.

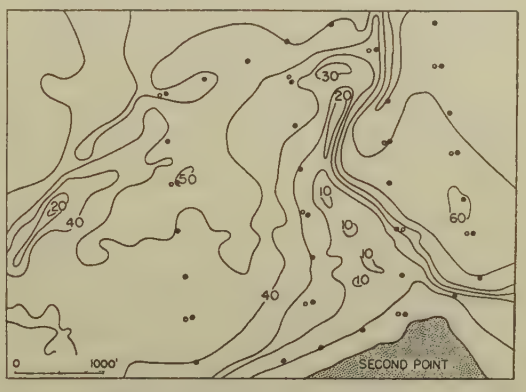

FIG. 2. Second Point Bar, Lake Mendota. Temperature (●) and current (○) observation stations.

standing-wave pattern. There was a tendency for divergence (hence, upwelling near the surface) wherever the wave was concave to the bar or to the shore (for example, point A on Fig. 3); convergence and sinking tended to occur wherever the wave was convex to the bar or to the shore (for example, point B on Fig. 3). Thus, the vertical velocities near the surface appear to have been dependent upon the speed of the surface current, which was ultimately governed by the wind. Probably the most noticeable feature is the strong, cellular pattern of rising and sinking water that developed with depth. This pattern occurred with all eastward currents, but the intensity of both the upwelling and sinking changed proportionally with the general

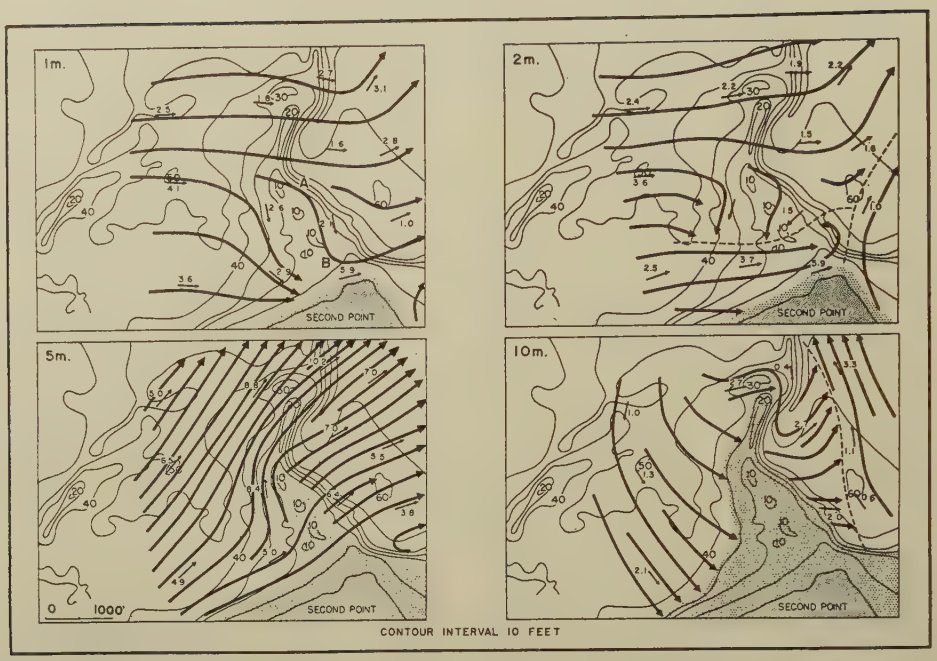

FIG. 3. Spaced streamlines for the 17–19 hour cruise, July 20, 1954. Current velocities are in centimeters per second.

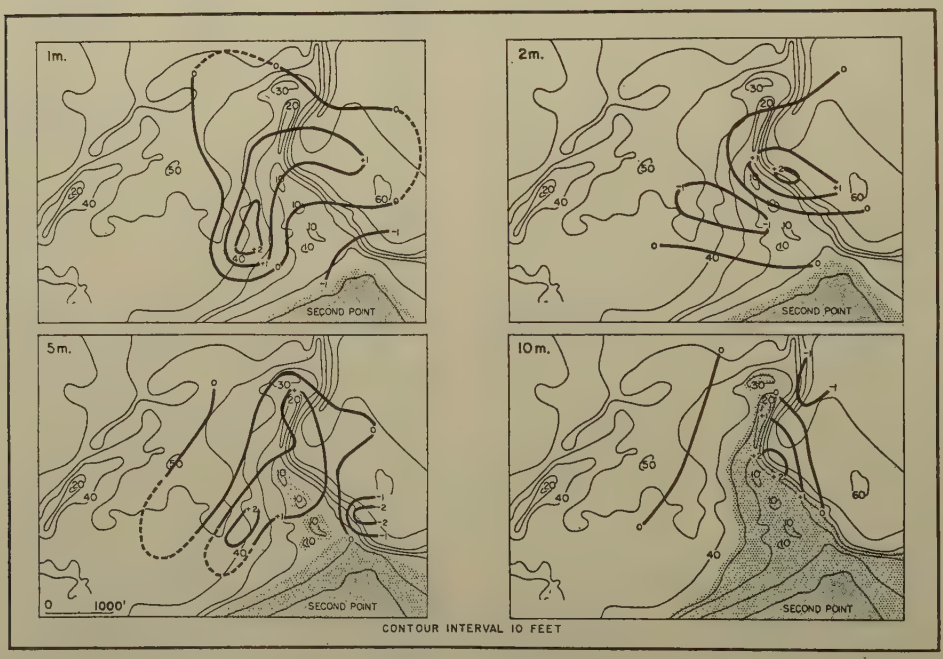

FIG. 4. Divergence for the 17–19 hour cruise, July 20, 1954. Values ($\times 10^{-4}$) are changes in area per second.

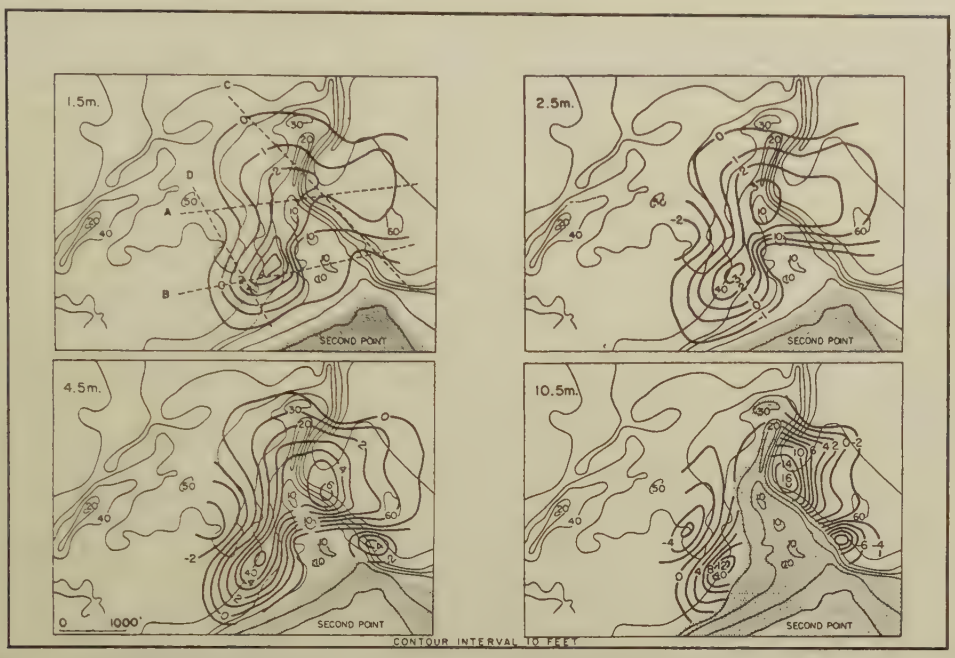

FIG. 5. Vertical velocity for the 17–19 hour cruise, July 20, 1954. Values ($\times 10^{-2}$) are in centimeters per second. Positive values indicate upwelling, negative values indicate sinking.

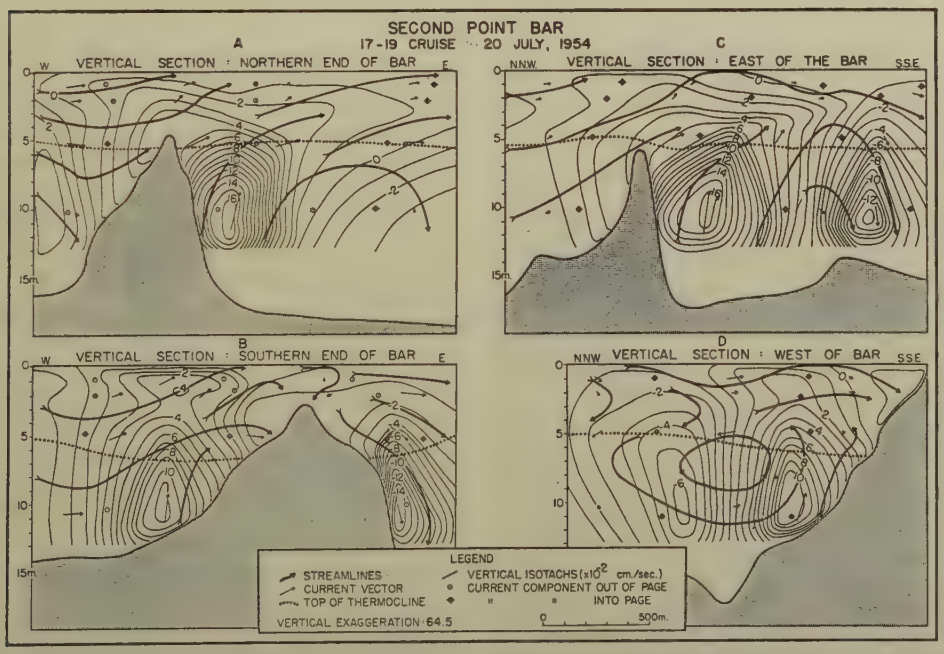

FIG. 6. Vertical cross sections through a vector field derived from the combination of the vertical and the horizontal velocity components. Section locations are shown on Figure 5.

flow speed. In general, the water rose on the windward side of the bar and sank on the leeward side. One exception was the area of upwelling centered in the bight on the eastern side of the bar. Reference to all the data shows that the areal extent and strength of this region of upwelling is a function of and proportional to the horizontal current speed.

Comparison of all maps with those with eastward flow patterns indicates that the location of the upwelling shifted southward along the eastern side of the bar as the wind became more northerly. While with westward flow the whole pattern nearly reversed itself, not completely, however, because of the asymmetric shape of the bar itself. As with eastward flow, the near-surface patterns of vertical velocities were not fixed but, apparently, again were dependent upon the current speed and standing-wave configuration.

Figure 6 presents four vertical cross sections for the 17–19 hour cruise of July 20, 1954. The vectors are determined from the horizontal and vertical components of the water flow and are exaggerated in scale with the vertical exaggeration of the cross section. As the water above the thermocline was practically isothermal, there was no stratification to restrict turbulent motion, whereas the water in the strongly stratified thermocline was very stable allowing only quasi-laminar flow within it. Yet only comparatively weak gross vertical flow occurred in the water above the thermocline, and the strongest vertical flow existed within the supposedly stable thermocline. From this we conclude that although there cannot be large turbulent velocities within stable layers, nevertheless, the whole body of water itself may undergo relatively rapid vertical displacement. This is reflected in the distortion of the top of the thermocline from an even surface to an undulating surface.

In section A of Figure 6 we can locate the area of upwelling discovered in the bight of the bar. It appears to have been caused by water rising from below in accord with the divergence at the surface as the water moved off the bar. Furthermore, the streamlines imply that here the water was actually moving in a clockwise rotation about a north-south axis. In section C of Figure 6 this same water appears to have been involved in a clockwise rotation about a northeast-southwest axis. If these two motions are treated as components and are combined into one unified movement, the upwelling appears to have been part of a helical flow as the water moved eastward away from the bar.

Another, though less developed, helical rotor can be located on the windward side of the bar. It was probably due at least in part to the obstruction effected by the bar to the oncoming water. This adds to the impression that the water in the thermocline was too stable to have risen and flowed over large obstacles, but for the most part must have flowed around instead.

A feature of this particular cruise was the "stress drop jet" of which Bryson and Bunge (1956) have written. It is best shown in section D of Figure 6. During the day there had been an onshore northeasterly wind which had driven more of the lake's epiliminial water against the shore; this depressed the thermocline. In the evening, the time of this cruise, the wind changed to westerly so that the force that had been holding the excess epiliminial water against the shore was released, and the solenoids created during the day were allowed to unwind. This figure shows that the resulting upwelling was pushing the thermocline up to its normal position. The displaced water above it was squeezed out at five meters as the so-called "stress drop jet."

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Length, Weight, and Age Composition of the Menhaden Catch in Virginia Waters[1]

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ABSTRACT

Major features of life history of menhaden (*Brevoortia tyrannus*) were deduced by examination of samples from commercial purse-seine and pound-net fisheries, and collections made by plankton nets, gill nets, and otter trawls. Menhaden are present in Virginia waters throughout the year. Spawning probably takes place in the ocean, and young move into Chesapeake Bay and its estuaries soon after hatching. As they grow, they move back down the estuaries toward the sea, so that, in most seasons, mean sizes of fish are progressively greater in a seaward direction. Few fish older than three years are taken in the commercial fisheries. The purse-seine fishery takes about 98 per cent of the total catch, operates from May to October, and exploits primarily fish in their third year of life in spring and early summer, and fish in their second year in late summer and fall. The pound-net fishery takes principally fish in their second year in spring and early summer, and fish in their first year in late summer and fall. Prior sampling by the pound-net fishery offers a method of forecasting purse-seine catches at least a year in advance. Ages of menhaden up to the end of the third year of life may be determined by the length-frequency method or by reading scales. Validity of the scale method has been established, and there is evidence that two groups of fish, characterized by their size at the time of formation of the first annulus, enter the Virginia fishery. Mean lengths at the end of each calendar year of life are approximately 125, 200, and 230 mm. Mean weights are about 30, 130, and 200 g. Spawning in the Chesapeake region probably is most intense in late fall and the following spring, associated with seasonal waves of emigration from and immigration to Chesapeake Bay. In this region maturity is reached at about one and one-half years of age.

INTRODUCTION

The Virginia waters of Chesapeake Bay and the ocean immediately outside the Capes yield a substantial part of the menhaden (*Brevoortia tyrannus*) catch of the United States. In 1955 over 300 million pounds of this important species, worth more than 3½ million dollars, were landed in Virginia ports, about one-sixth of the entire U. S. menhaden catch.

Considering the great importance of the menhaden fishery, which lands each year a greater weight of fish than any other fishery in the Western Hemisphere, it is surprising how little is known of the habits of the species. Existing knowledge was summarized by Ellison (1951), who cited only 27 references, and only a few additions to this literature have appeared since 1951. Lee (1952) gave a summary of recent developments in the industry. In the meantime, however, a major investigation of the menhaden resource has been inaugurated by the U. S. Fish and Wildlife Service. This work, of which the first published report has appeared recently (June 1958), is adding immeasurably to our knowledge of the fishery, and of the habits of this important species.

The investigations reported here began in 1954, when the task of learning something of the size and age-composition of menhaden in the commercial catch was assigned to R. T. Oglesby, a graduate student at the Virginia Fisheries Laboratory. The results of his study (Oglesby 1955) were sufficiently promising that we decided to continue the investigation in limited fashion. Since 1954, most of the samples have been examined by the senior author, with help at various times by

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Susie V. Floyd, E. C. Ladd, Sung Yen Feng, W. H. Massmann, J. P. Whitcomb, and C. E. Richards. The junior author, A. L. Pacheco, collected many of the samples and also assisted with analysis of some data.

MATERIALS AND METHODS

In 1954 samples were taken once each week from purse-seine catches landed at Reedville, Virginia, and from pound-net catches in the York River. Fork length in millimeters, weight in grams, and sex were recorded, samples of scales were taken, and observations were made on gonad condition. In 1955, 1956, and 1957, only pound-net catches were sampled, and in the last two years scales were taken usually only from larger fish, the age of which could not be estimated readily on the basis of size. All fish were examined fresh.

Scales were soaked in water, cleaned between the fingers, and mounted dry between two slides. In 1954 they were examined by projecting the image downward onto a table with a Promi projection microscope, and measurements were recorded on index cards. Later it was found more convenient to examine scales under low power of a dissecting microscope, and to measure growth increments with an eyepiece micrometer.

Previous authors have presented length-frequency distributions of menhaden, and it was of interest to compare our measurements with theirs. Often this was difficult, for the method of measurement was not described, and we did not know whether standard length, fork length, total length, or some other unit had been used. Fortunately, most of these authors reported also the weight of the fish, and this allowed us to decide which length measurement was used. By measuring fork length and total length on a sample of Chesapeake Bay menhaden we determined their relationships to weight and concluded that Hildebrand and Schroeder (1928) and Goode (1879) used total length, namely, length from tip of snout to end of caudal fin with lobes fully extended on the mid-line of the body. Early authors also recorded their measurements in inches and weights in ounces. When these measurements were converted to metric units class-intervals were irregularly spaced, and it was necessary to smooth frequency distributions twice by moving averages of three to restore some semblance of regularity to the data.

In discussing age of fishes, confusion often arises because terminology has not been well defined. Menhaden apparently spawn from fall through spring in the Chesapeake region, and it is convenient to take January 1 as the date of birth, so that the biological year coincides with the calendar year. In referring to year of birth, we have assigned to the 1955 year class all menhaden born in the spawning period that began in the fall of 1954 and extended into the spring of 1955, and have designated other year classes accordingly. Formation of annual markings on the scales is essentially complete by the end of May, but in their first year most menhaden probably do not form an annulus, for by the first May of their lives many of them have not yet formed scales. Therefore, young fish may be considerably more than a year old when the first annulus becomes apparent. Confusion is avoided by adopting the convention discussed by Hile (1948) in which a virtual annulus at the edge of the scale is credited from January 1 to the date on which the new annulus is completed. Thus a fish with no annuli will belong to age-group 0 until January 1, then automatically will be advanced to age-group I, and so on. Like all systems of classification, this requires exceptions, for a menhaden born early in the breeding season may have formed scales by January 1, when it is only a few weeks old. Obviously, such a fish should not be classified in age-group I at such an early age, and error can be avoided by reference to the size of the fish and dimensions of its scales.

SIZES OF MENHADEN CAUGHT IN VIRGINIA

Menhaden are present in Virginia waters of Chesapeake Bay at all times. Seasonal variations in size of fish are illustrated in Figure 1. In early spring the first pound nets, set primarily to catch shad, took small numbers of large menhaden, and as

the fishing season progressed relative numbers of large fish decreased and small menhaden appeared more frequently. In winter small fish sometimes were caught in experimental trawls in deeper waters of the Bay, especially in cold weather, and larger fish were taken in estuaries with gill nets. Occasionally, even postlarvae were captured in midwinter. In the estuaries postlarvae occurred in large numbers in spring, and juveniles usually were common in summer.

Sexual dimorphism in length

Westman and Nigrelli (1955) found that female menhaden were slightly larger than males of the same age in New York waters. Tester (1937), Phillips (1948), and Clark and Phillips (1952) demonstrated similar sexual dimorphism for other clupeoid species. Female menhaden in Chesapeake Bay purse-seine samples also were somewhat larger than males (Table 1), but the difference was not sufficient to warrant separation of sexes in comparing samples. In pound-net samples no consistent sexual difference in length was found, and length-frequency distributions were more regular if sexes were not separated. It is probable that in menhaden, as in Pacific sardines (Phillips 1948), sexual dimorphism in length is apparent only in older fish. There appears to be no sexual dimorphism in weight of fish of the same length.

Purse-seine fishery

Random samples of purse-seine catches were taken weekly at Reedville, Virginia, from April 21 to October 25, 1954. These catches were made at many different localities in Chesapeake Bay, and some in the ocean outside the Virginia Capes (Fig. 2). Fishermen say that menhaden schools usually consist of fish of uniform size, and that fish taken in the ocean usually are larger than those taken inside the Bay. Our sampling did little to confirm these observations, although in the early part of the season there seemed to be relatively more young fish in the Bay than in the ocean. To decide this point would require many more samples than we were able to examine.

Length-frequency distribution patterns can be demonstrated most easily by grouping samples by months. Although information on the magnitude of purse-seine landings was available, it did not seem advisable to weight samples according to the amounts landed, for weekly samples of 100 fish represent only an infinitesimal part of the catch. Such infrequent sampling cannot necessarily be considered representative of length composition of the week's landings, for there is evidence that menhaden in Chesapeake Bay school differentially according to size. Nevertheless, the unweighted samples, grouped by months (Fig. 1), illustrate in a general way several important features of the length composition of purse-seine catches: (1) two principal size groups of fish were caught; (2) the larger size group was dominant early in the season, and declined in relative abundance in summer and fall; (3) the smaller size group first entered the fishery in strength in July, and dominated catches thereafter; (4) modes representing these two length-groups on length-frequency polygons moved to the right as the season advanced.

Pound-net fishery

Although pound nets take only a small part of total menhaden landings in Virginia, this catch is worth investigating for several reasons. Pound nets can be sampled readily from Gloucester Point, whereas arrangements to sample the purse-seine fishery are complicated by distance. It is necessary first of all to determine whether pound-net catches differ from purse-seine catches in any essential characteristics, and if so, whether these differences are constant. If these conditions are satisfied, then an analysis of pound-net catches may provide useful information on the purse-seine fishery, and hence on stocks available to the commercial fishery, with much less expenditure of effort.

Concurrently with investigation of purse-seine catches in 1954, the pound-net fishery in the Gloucester Point region was examined. Samples were obtained weekly from a series of nets in the lower York River, and oc-

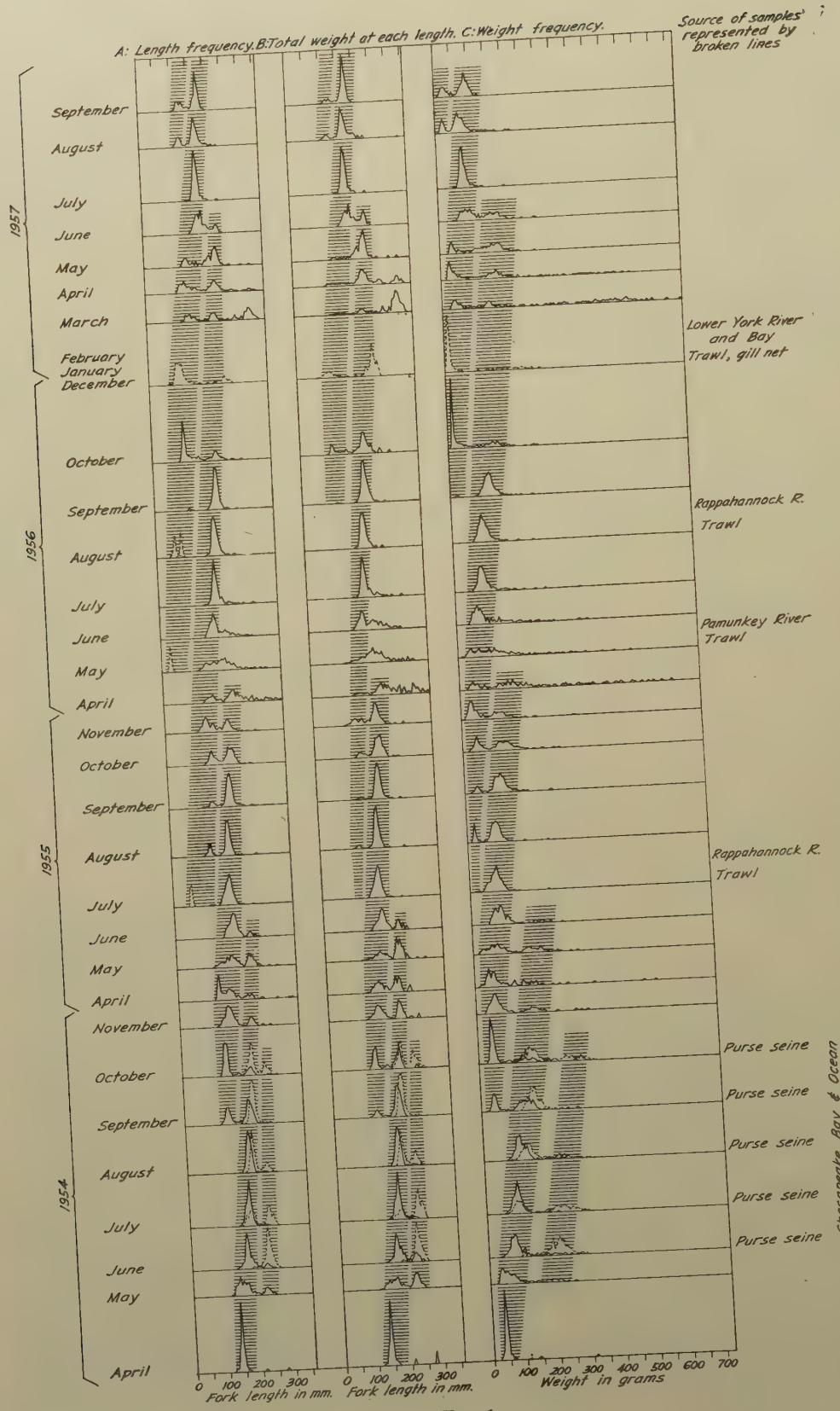
A: Length frequency. B: Total weight at each length. C: Weight frequency.
Source of samples represented by broken lines
1957
September
August
July
June
May
April
March
February
January
December
1956
October
September
August
July
June
May
April
1955
November
October
September
August
July
June
May
April
1954
November
October
September
August
July
June
May
April
Lower York River and Bay Trawl, gill net
Rappahannock R. Trawl
Pamunkey River Trawl
Rappahannock R. Trawl
Purse seine
Purse seine
Purse seine
Purse seine
Purse seine
Chesapeake Bay & Ocean
0 100 200 300
Fork length in mm.
0 100 200 300
Fork length in mm.
0 100 200 300 400 500 600 700
Weight in grams

FIG. 1

TABLE 1. *Length frequency distributions of sexes in samples of menhaden from the Virginia purse seine catch, September to October 1954*

Fork length mm	Sept. 2		Sept. 13		Sept. 27		Oct. 4		Oct. 25	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
185	2	..	..	..	..	..	..	..	..	..
190	4	8	..	..	..	1	3	..	4	..
195	11	10	5	5	..	..	3	2	2	2
200	12	17	12	7	2	3	3	6	1	9
205	8	13	18	18	8	7	7	5	9	6
210	2	7	7	13	20	11	11	17	14	10
215	..	1	4	5	12	17	11	20	4	12
220	..	..	1	1	4	6	2	4	3	3
225	..	..	..	..	2	3	..	2	..	2
230	..	..	..	..	..	..	..	..	..	1
235	..	..	..	..	..	1	..	..	..	..
240	..	..	..	..	..	1	..	..	..	..
245	..	..	..	..	..	..	..	..	..	..
250	..	..	..	..	..	..	..	..	..	..
255	..	..	..	..	..	..	..	..	..	..
260	..	..	..	..	..	1	..	..	..	..
Means	198.3	200.4	204.6	205.9	211.5	214.1	207.6	211.0	206.9	209.8

casionally also from nets in Chesapeake Bay near York Spit. This sampling program has been continued through 1957. Length-frequency distributions, summarized by months, are illustrated in Figure 1. In combining data the samples were not weighted, for accurate information was not available on pound-net landings of menhaden. The curves in Figure 1 are probably accurate as to numbers of modal length groups and their positions, but they do not necessarily reflect relative importance of various length groups in the fishery from month to month or from year to year. Included also in Figure 1, for comparison, are length-frequency distributions of samples from purse seines, experimental trawls, gill nets, minnow seines, and plankton nets, represented by broken lines.

Figure 1 shows that at least three distinct length groups of fish were prominent in the pound-net fishery in the vicinity of the York River. In spring (March to May) large fish were relatively numerous, and in March and April many of the fish exceeded 250 or even 300 mm in fork length. The principal modal length groups, however, lay between 125–150 mm and 210–230 mm. This would be especially apparent if samples were weighted according to magnitude of the catch, for larger fish appeared in the fishery for a relatively short time, and only in relatively small numbers. Larger fish disappeared rapidly from catches through spring and early summer, but the modal length of the group of smaller fish increased. By August or September another modal group appeared, with average length of about 100–135 mm, and the adjacent mode had shifted meanwhile to about 175–195

FIG. 1. Percentage frequency distributions by months of fork lengths (*A*), total weights of fish in each 5-mm length group (*B*), and frequency distributions of weights (*C*) of menhaden samples from Chesapeake Bay, 1954–1957. Polygons enclosed by solid lines represent samples from pound nets, those enclosed by broken lines represent samples from purse seines (1954) and plankton nets, trawls, and gill nets (1955–1957). Shaded zones emphasize the progression of modal groups to the right as the fish grow and illustrate the continuity of occurrence of dominant size groups in the fishery in successive years. Vertical distances between horizontal bars represent 2 units on the percentage-frequency scale. Series *A* represents the conventional way of expressing such data in scientific publications, series *B* presents the size groups in their true proportions by weight as measured in the fishery, and series *C* achieves maximum separation of age groups.

FIG. 2. Virginia waters of Chesapeake Bay and its tributaries showing location of inshore (black circles) and offshore (open circles) pound nets from which samples were taken in 1954–1957, and locations of purse-seine catches sampled in 1954 (shaded circles).

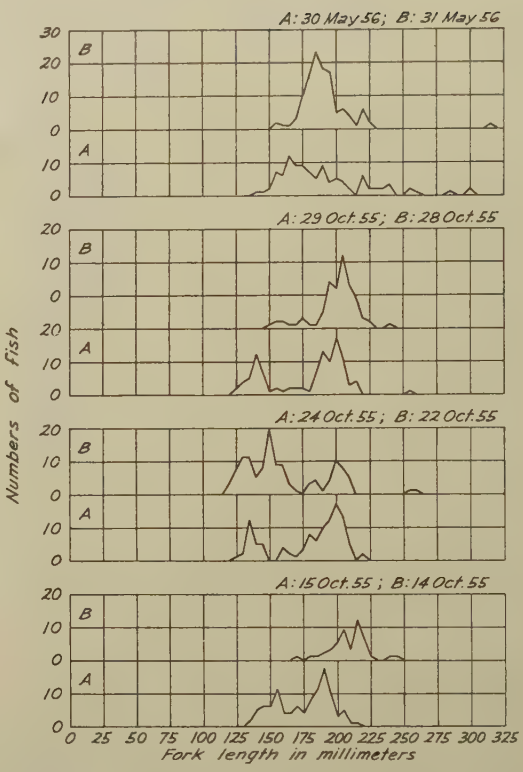
A: 30 May 56; B: 31 May 56
A: 29 Oct. 55; B: 28 Oct. 55
A: 24 Oct. 55; B: 22 Oct. 55
A: 15 Oct. 55; B: 14 Oct. 55
B
A
Numbers of Fish
0 25 50 75 100 125 150 175 200 225 250 275 300 325
Fork length in millimeters

FIG. 3. Length-frequency distributions of menhaden caught in inshore (*A*) and offshore (*B*) pound nets in the York River and Chesapeake Bay.

mm. Larger fish at this time were rare, but in late fall they began to appear in catches in relatively larger numbers.

Local variations in length

It has been suggested already that menhaden caught by purse seines in the ocean usually are larger than those taken inside the Bay. Figure 1 shows that in 1954 fish in the purse-seine catch were larger than those caught in pound nets. In most months in 1954 three distinct modal length groups were present in the fishery, but the left-hand mode usually was absent from purse-seine catches and the right-hand mode absent from pound-net landings. Furthermore, fish within the same modal group were larger in purse-seine catches.

There also were fairly consistent differences in length-frequency distributions of menhaden caught in pound nets in rivers and in the open Bay. This is illustrated by measurements of several samples taken on approximately the same dates from the lower York River (black circles in Fig. 2) and from Chesapeake Bay (open circles). Positions of the major modes in length-frequency polygons of samples from the Bay (Fig. 3) were almost always to the right of those derived from the river catch.

Farther up the estuaries even smaller fish were found. Massmann, Ladd, and McCutcheon (1954), in brackish and fresh waters of the Rappahannock in June and July 1951, took young whose average length was about 41 mm. In late September the average length of young caught in brackish waters of the same river was 94 mm. On May 9, 1956, a sample from Station P33 on the Pamunkey River had an average length of 31 mm. Three weeks later, a sample from Station P50, 17 miles farther upriver, was only 26 mm in average length, although the young fish are growing rapidly at this time. A better illustration of the gradient in length of fish up and

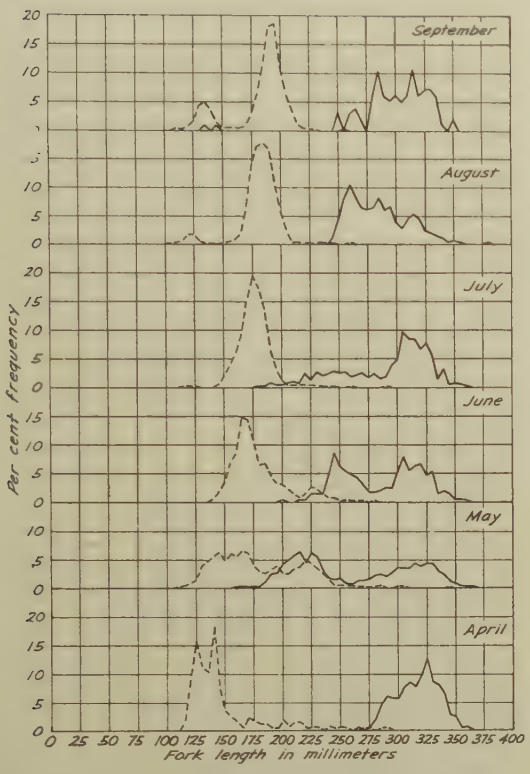

FIG. 4. Length-frequency distributions of menhaden sampled by Westman and Nigrelli (1955) in New York and New Jersey waters (solid lines), and by the Virginia Fisheries Laboratory in comparable months in Chesapeake Bay, 1954–1957 (broken lines).

down stream is given by two samples of young illustrated in Figure 1 for August 1956, both taken in the Rappahannock River on August 29. At Station R50 the average length was about 60 mm. At Station R45, five miles downstream, the average length was almost 80 mm.

Westman and Nigrelli (1955) have presented information on length-frequency distributions of menhaden caught in New York and New Jersey waters. In 1948 and 1949 two major length groups of fish appeared in samples from the commercial catch, with modes at about 250 and 320 mm. In May 1953 a dominant group of smaller fish, with a modal length of about 220 mm, entered the fishery. Assuming that these length-groups are always the most important groups in the middle Atlantic fishery, it would appear that at any given time menhaden caught in the Chesapeake area are smaller.

Dr. Westman has kindly permitted us to examine his original data. To compare these with Chesapeake Bay pound-net samples, we have grouped measurements of fork length in 5-mm class intervals. Length frequency polygons are presented in Figure 4. Obviously menhaden caught in waters off New Jersey and New York were larger and probably older than those in Chesapeake Bay pound-net catches. It is possible, of course, that the difference in length was caused by a change in length and age composition of menhaden stocks in the period since Westman's samples were taken. In support of this possibility is the observation by Westman and Nigrelli (1955) that large numbers of fish in their second year of life appeared in the 1953 fishery in New York and New Jersey, but none in 1948 or 1949. On the other hand there are the observations of fishermen, who say that they consistently catch larger menhaden in northern waters, and that fish caught in Chesapeake Bay in the past were just as small as they are now. Measurements recorded by Hildebrand and Schroeder (1928), which we have converted from inches to metric units and smoothed twice by a moving average of three to eliminate irregularities caused by the conversion, also were similar in range of lengths and positions of modes to pound-net catches in recent years (Fig. 5). It is interesting also that a few fish from Noank, Connecticut, measured by Goode (1879) were similar in length to the three- and four-year-old fish reported by Westman and Nigrelli.

Because there was so little overlap in fork lengths of Chesapeake and New York fish (Fig. 4), it was not possible to discover if fish of the same age in the two places differed in length. May was the only month in which substantial numbers of fish lay within the same length range in both localities, but the New York data were based on a single sample, which, in view of the local variations in size already demonstrated, may not be representative. There appears to be no real difference in length between the two modal groups.

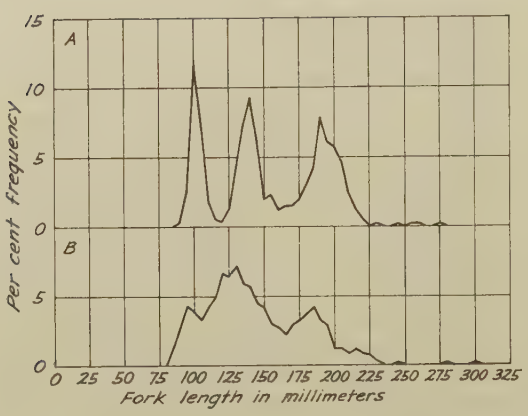

FIG. 5. Length-frequency distributions of menhaden sampled by Hildebrand and Schroeder (1928) in October in Chesapeake Bay (*B*), and by the Virginia Fisheries Laboratory in the same month in 1954–1957 (*A*). The two left-hand modal groups in the upper polygon (*A*), represent the two size-groups of yearling fish that show up periodically in Chesapeake Bay.

AGE DETERMINATION

Estimation of age from length distribution

The rather distinct and uniform tendency to segregate into discrete size groups, in a pattern that shifts regularly with seasons and repeats itself year after year, suggests that successive modes in length-frequency distributions of menhaden represent separate age groups of fish. In spring catches the two principal length groups lay between 100–150 mm and 200–250 mm (Fig. 1). As Walford and Mosher (1943) have pointed out, for most fishes the rate of growth in length declines steadily with age. Therefore, if one or more groups of younger menhaden were to exist at this time, they should be separated from the left-hand mode and from each other by distances greater than that separating the two adjacent modes that dominate pound-net and purse-seine catches. For this reason it would appear that only one smaller size group, at the most, could occupy the space to the left. Small menhaden caught in rivers in July 1955 and May and August 1956, in York River pound nets in October 1956, and trawled in Chesapeake Bay in January and February 1957, seemed to belong to this initial size group, some of which in May 1956 were still in the post-larval stage. If the mid-point of the spawning period falls in winter, then these were fish in their first year of life, which reached lengths of about 100–150 mm at the end of their first year. This matches well with the modal group of small fish in the pound-net catch in spring, which in 1954 showed up in purse-seine catches in strength in July. The position of this mode moved steadily to the right through summer and fall, and by October or November it reached 190–210 mm at approximately the end of the second year of life. This merged with the second mode in the pound-net catch the following spring, which as mentioned above lay between 200–250 mm. Progression of this length group to the right during the third year of life can be followed best in the 1954 purse-seine samples. Menhaden older than two years did not appear prominently in pound-net catches except in early spring, at a time when abundance of menhaden, other than juveniles, in Chesapeake Bay and its estuaries appeared to be at its lowest.

Size composition of fish catches may be presented in several different ways, each of which has its own peculiar advantages. By far the most commonly-used method is to group body lengths by some convenient class-interval, and to present individual frequencies as fractions of the total number of fish in the sample. When plotted on squared paper this treatment gives a series of frequency polygons, as in Figure 1A, in which positions of dominant size groups and their seasonal shifts in response to growth and other factors are more or less clearly defined. Body length is the most convenient criterion of size because it can be obtained quickly and accurately under a wide variety of working conditions, and often is the only satisfactory measurement that can be made at sea. The principal disadvantage is that fishery catches usually are measured by weight, and length-frequency distributions therefore fail to present a true representation of biomass.

If weights have been recorded individually, they may be summed for each category of body length, and expressed as

fractions of total sample weight, as in Figure 1B. This presents modal size groups in their true relation with respect to distribution of weights of fish within the sample. A further refinement, and one that is necessary if a picture of size composition of the season's catch is to be derived, is to weight each sample in proportion to total catch in the period the sample represents. This could not be done for the samples herein described, for catch records were not obtained. This refinement is not necessary or even desirable when the chief interest lies in size distribution and growth rates.

The same information can be treated in yet another way, in the form of frequency distributions by weight (Fig. 1C). This gives better separation of dominant size groups of small fish, but because weight is a parabolic function of length the modes become more diffuse toward the right.

On the basis of length- and weight-frequency distributions of menhaden caught in the principal commercial gears and in various other collecting devices we have assumed that most fish caught in pound nets during the period 1954–1957 were in their second year of life, that in spring some older fish were taken, and that in fall menhaden approaching the end of their first year of life appeared prominently in the catch.

Under the same assumptions, but based on samples from the 1954 fishery only, menhaden in their third year dominated the early part of the purse-seine season, but declined rather steadily in relative importance as the season advanced, to be replaced by fish in their second year of life. It must be recognized, of course, that although this hypothesis may be generally correct, it is probable that with increasing age more and more admixture of age-groups of fish goes undetected by the length-frequency method.

Estimation of age from scales

Rush (1952) and Westman and Nigrelli (1955) have reported that ages of menhaden can be determined from markings on the scales, and their conclusions appear to be in fair agreement. Rush, however, examined a very small sample of fish, and Westman and Nigrelli made no attempt to test the reliability of their criteria, other than to observe that independent readings by two persons gave very close agreement. They also pointed out that although some scales "appeared to exhibit clearly defined annuli," most "could be read with some difficulty," and "the third, fourth, and fifth annuli on the scales of the larger fish were often so close together near the periphery that accurate age diagnosis was difficult."

Walford and Mosher (1943), in their masterly study of methods of age-determination in the Pacific sardine (*Sardinops caerulea*), proposed certain criteria by which an annulus could be identified and its validity as an annual marking confirmed. We have found that markings on menhaden scales are sufficiently similar so that essentially the same criteria can be adopted. Other marks of somewhat similar appearance, termed false annuli, are common on scales of many fishes. For these also the criteria of Walford and Mosher were applicable to menhaden scales.

Among other markings on menhaden scales are transverse grooves, deep grooves running dorso-ventrally across the scale. The most posterior groove extends across the scale from margin to margin, and for convenience in scale measurement this has been termed the base line. Almost always the grooves are less distinct towards the imbedded portion of the scale, and appear to degenerate into series of broken lines which become fainter and fainter until they seem to disappear completely on the anterior third or quarter of the scale. Striae cover the sculptured portion, appearing in projected image as fine lines running dorso-ventrally across the scale. These are caused by small wave-like upturnings of the scale surface. All measurements of projected images of scales originated at the base line, which varied in width and distinctness, but which could be resolved by careful focusing into a fairly narrow and clearly-defined marking.

The first four criteria adopted by Walford and Mosher for recognition of an annulus on scales of *Sardinops caerulea*, and all

TABLE 2. *Frequency distributions of width of marginal increments on scales of age-group I menhaden from pound-net samples from the York River, 1954*

Width of marginal increment in arbitrary units	Frequency of occurrence						
	21 Apr.–10 May	17 May–31 May	7 June–21 June	28 June–12 July	19 July–2 Aug.	9 Aug.–23 Aug.	31 Aug.–13 Sept.
2	36	3	..	..	..	..	..
5	73	39	2	..	..	..	..
8	84	90	26	..	..	..	..
11	31	68	59	3	..	..	..
14	4	16	83	13	2	..	1
17	..	6	61	37	12	1	1
20	..	..	21	72	38	17	3
23	..	..	7	68	75	28	6
26	..	..	2	57	61	51	21
29	..	..	..	26	49	48	33
32	..	..	..	8	26	50	38
35	..	..	..	4	12	44	40
38	..	..	..	1	7	27	40
41	..	..	..	1	..	12	26
44	..	..	..	..	3	5	26
47	..	..	..	..	1	..	23
50	..	..	..	..	1	2	8
53	..	..	..	..	..	2	9
56	..	..	..	..	..	..	10
59	..	..	..	..	..	..	4
62	..	..	..	..	..	..	1
65	..	..	..	..	..	..	1
68	..	..	..	..	..	..	..
71	..	..	..	..	..	..	1
Mean width	6.6	9.0	14.7	22.6	26.3	30.7	37.8

those describing a false annulus, are arbitrarily-defined characteristics, based on experience with scale reading and some knowledge of menhaden scales, that need no further elaboration. The fifth criterion, however—that one and only one annulus be formed each year—must be examined and established for menhaden scales. If it can be shown, for fish having an equal number of marks conforming to the first four criteria, that the marginal increment of scale material outside the last acceptable mark increases in width throughout the year, and that most, or preferably all, such marks are formed during a definite season, then these markings can reasonably be called annuli.

Frequency distributions of marginal increment widths in scales of age-group I fish from 1954 pound-net catches are given

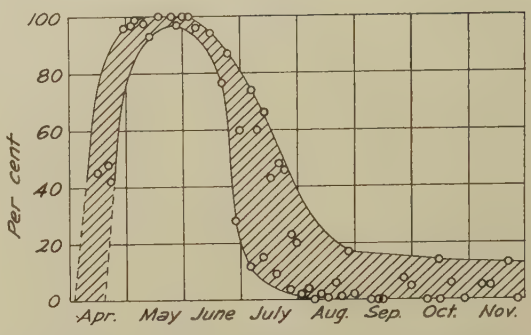

FIG. 6. Seasonal variation in relative numbers of age-group I menhaden from Chesapeake Bay bearing narrow marginal growth increments at the edge of the scale, 1954 and 1955.

in Table 2. These scales were read by Oglesby. Frequencies were grouped in classes of three arbitrary measurement units, and samples were grouped by three-week intervals. Statistical analysis is not necessary to demonstrate or to prove the steady and significant increase in width as the season advances. Scales of menhaden from 1955 samples, examined by McHugh, exhibited a similar growth pattern. By selecting an arbitrary line of division to separate the series of measurements into two groups of equal numbers having "narrow" and "wide" marginal increments, the patterns illustrated in Figure 6 were derived. From this it can be deduced that annulus formation in 1954 and 1955 took place in spring, that all annuli of fish in their second year of life had been formed before the end of May, and that scales grew out beyond the annulus for the rest of the season. Scale measurements from age-group I fish were most satisfactory to demonstrate validity of the annulus, for fish of this age were available in pound-net catches throughout the season. Patterns were similar for age-group II fish, but these older fish were not available to pound nets in very great numbers after mid-summer.

Comparison of methods of age determination

Assuming that the major modes of frequency distributions of fork length represented successive age groups of menhaden, and that the left-hand modal group, which appeared in pound-net catches in fall, represented fish of about one year in age,

we have concluded that the commercial menhaden fisheries of Virginia usually exploit three groups of fish, which in fall are respectively about one, two, and three years old.

Examination of scales has confirmed these tentative age determinations based on lengths. Successive length groups have been identified as adjacent age groups, and relative frequencies and biomass of various ages can be estimated rather closely from lengths alone. For specific age determination of individual fish, of course, the scale method is necessary, for overlap of succeeding age groups probably increases rapidly with age. However, accuracy of age determination also decreases in older fish, for spacing between annuli decreases with age, and arbitrary criteria set up for annulus identification are more difficult to satisfy.

Catch records were made available to us by the factory which processed the purse-seine catches sampled in 1954. Using these figures to weight the sample length frequencies, a rough approximation in view of the small number of samples, we have concluded that 70 per cent of fish in the 1954 purse-seine catch were in their second year of life, and most of the remaining 30 per cent were in their third year. No weighted estimate of the age distribution of the pound-net catch could be made, but a simple combination of all the samples examined in 1954 gave 89 per cent in the second year of life, 8 per cent in the third, and the remaining 3 per cent in the first or fourth year. A similar combination of 1955 readings gave 77 per cent in the second year, 14 per cent in the third, 8 per cent in the first, and the remaining one per cent in the fourth, fifth, and sixth years of life. Figure 1 suggests that relative abundance of larger fish increased throughout the period of sampling, and this confirms information received from F. C. June (personal communication) that the 1951 year class of menhaden was unusually strong. This possibility was suggested also by Massmann *et al.* (1954) from observations of juvenile menhaden in 1951.

RATE OF GROWTH

Because menhaden school so precisely according to size, and because such distinct gradients in size of fish exist up and down the estuaries, within the Bay, and probably also in the ocean, no precise picture of growth rate can be traced, if indeed the concept of a single rate of growth can be applied to such a wide-ranging species. The existence of two distinct groups among age-group I fish, characterized by different lengths at time of formation of the first annulus, and the tendency toward a bimodal length-frequency distribution in some samples of young fish, suggest that growth rates differ according to circumstances. However, a general conception of growth rates can be derived from length- and weight-frequency distributions, as illustrated by the shaded bands in Figure 1, and from ages deduced from scales.

Growth in first year of life as deduced from scales

Oglesby (1955) found that the mean distance from base line to first annulus in age-group I menhaden from pound-net catches decreased significantly throughout the fishing season. He suggested that this could be brought about by differential recruitment of fish with respect to size at time of annulus formation. More detailed examination of the first year's growth pattern on scales of the 1955 samples suggested that the phenomenon was caused by the existence of two distinct groups of fish, characterized by quite different growth patterns. In the arbitrary units of scale measurement used in 1955, scales of these two groups exhibited modes at 27 and 39 units respectively in frequency distributions of radius of the first year's growth zone. Positions of these modes were quite constant throughout the fishing season, but the longer radius dominated spring and early summer catches, and the narrower zone was dominant in late summer and fall (Figs. 7 and 8).

Apparently two rather distinct groups of menhaden, characterized by their lengths at the time the first annulus was formed, entered the Chesapeake Bay fishery in 1954 and 1955. Modal lengths of these fish

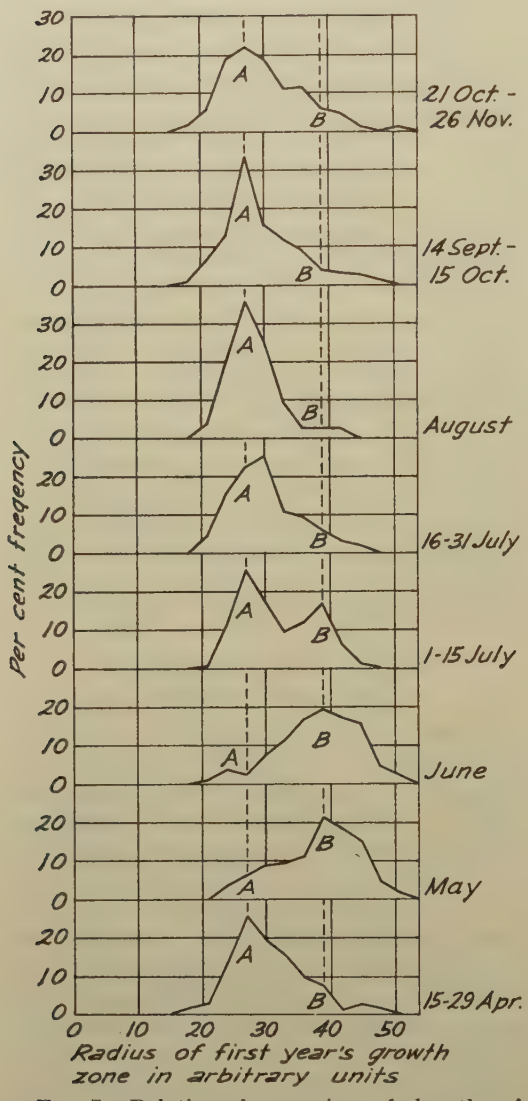

FIG. 7. Relative frequencies of length of radius of first year's growth zone on scales of age-group I menhaden in samples from pound-net catches in Chesapeake Bay in 1955.

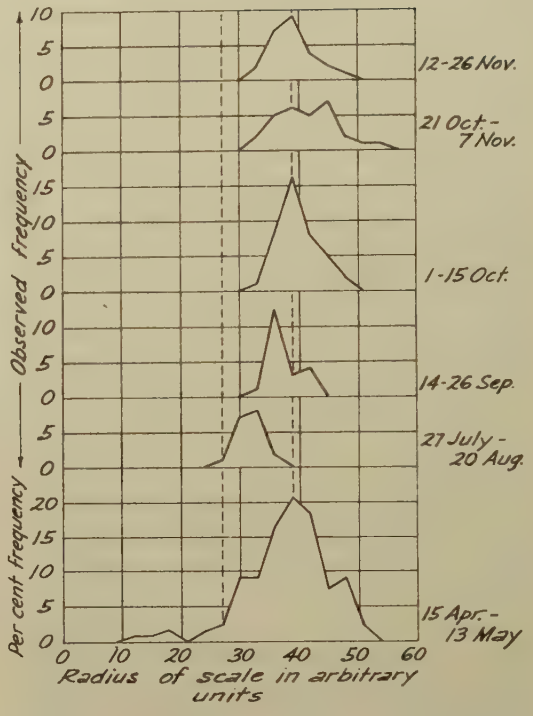
Per cent frequency ← Observed frequency →
12-26 Nov.
21 Oct. - 7 Nov.
1-15 Oct.
14-26 Sep.
27 July - 20 Aug.
15 Apr. - 13 May
0 10 20 30 40 50 60
Radius of scale in arbitrary units

FIG. 8. Relative frequencies of length of scale radius in menhaden in which the first annulus had not yet appeared in samples from pound-net catches in Chesapeake Bay in 1955.

at annulus formation were 100–120 mm and 140–160 mm. For convenience we have called these groups A and B, respectively. In early spring, fish with one annulus were predominantly group A (Fig. 7), but these were mostly in their third year of life, about to form their second annulus. By May, most fish with one annulus belonged to the succeeding year class and were in their second year, having just formed their annulus. These belonged predominantly to group B, which remained the most important group among age-group I fish until mid-July. Thereafter, group A dominated this age group of fish.

Measurements of the radius of menhaden scales in which the first annulus had not yet appeared provided additional information on seasonal occurrence of group B (Fig. 8). In spring the modal scale radius was 39 units. This was the same group that appeared as age-group I fish in May (Fig. 7). Age-group 0 menhaden of the succeeding year class appeared in the catch in late summer, with scales already larger than group A, and by October (Fig. 8) the mode had progressed to the position typical of group B.

If the pattern observed was typical, as its repetition in three successive year classes appears to testify, group B is important in Virginia's pound-net fishery for about a year, from its first appearance as a newly-recruited age group in late summer until it is

superseded by group A in mid-summer the following year. The sequence can be traced also in Figure 1, which illustrates how group B is recruited as a sharply-defined modal group, which becomes broader and more diffuse the following year as group A joins it, then becomes narrower and more sharply-defined again as group A assumes a dominant role.

The possibility remains that the decline in mean radius of the first year's growth zone is a natural consequence of recruitment of larger, faster-growing fish early in the season and that smaller, younger, or slower-growing fish enter the fishery at progressively later intervals as they reach appropriate sizes. Opposing this explanation is the consistent position of group B at a modal size of 39 units from fall to mid-July of the following year, the bimodal distribution of lengths of the first year's growth zone in July, and the sudden and permanent shift to a modal width of 27 units in late July. The phenomenon merits further investigation.

Growth in weight

Whereas apparent lengths at the end of each calendar year of life of menhaden caught by pound nets in Chesapeake Bay were approximately 125, 200, and 230 mm, mean weights were about 30, 130, and 200 g, respectively. For this reason, as mentioned previously, frequency distributions of weights should be even more useful than lengths as an indirect method of age determination; yet this method has been used rarely in fishery work. As illustrated in Figure 1C, weight-frequency distributions are particularly useful to identify the group of small fish that enters the pound-net fishery in late summer and fall. The position of this mode when the group first appears, its progress to the right through spring and summer, and its relation to the origin and to the next adjacent mode, offer convincing indirect evidence that these fish were in their first year of life when they entered the fishery, and that the adjacent mode at about 125 g represented fish one year older.

TABLE 3. *Length-weight relationship of menhaden caught in spring and fall*
Combined data for 1954–1956 inclusive, from pound nets in the lower York River, Virginia.

Fork length mm	April–May		September–October	
	Mean weight in grams	Number of fish	Mean weight in grams	Number of fish
120	25.1	9	27.7	26
125	28.2	16	33.6	41
130	32.7	65	37.4	86
135	36.6	83	41.7	91
140	40.4	127	46.7	73
145	45.2	108	51.2	28
150	51.3	80	56.0	12
155	57.1	100	65.3	7
160	63.2	80	71.5	8
165	70.6	69	78.0	9
170	75.1	66	84.0	27
175	84.9	54	91.6	53
180	93.0	42	97.8	122
185	103.4	50	105.6	172
190	110.9	47	114.8	267
195	119.6	58	125.3	291
200	130.2	40	135.3	200
205	140.4	44	145.8	111
210	147.3	45	157.7	65
215	159.7	47	164.8	27
220	169.6	53	188.3	12

As illustrated in Table 3, menhaden in the same length range were consistently heavier in late summer than in spring. From April to November this difference was of the order of 5 to 10 g in fish 125 mm long, and 10 to 15 g at 200 mm, approximately a 20 per cent increment at the smaller size, and 10 per cent at the larger. Several possible causes of such a weight differential suggest themselves. (1) Menhaden may become fatter and heavier through increased feeding in spring and summer, and through maturation of the gonads. (2) Because menhaden grow rapidly in early summer, fish of a particular length caught in fall are about a year younger than those caught in spring, and fall fish probably are the largest members of their age group. Thus change in weight may simply mean that the larger fish in a year's brood are heavier at a given length than the smaller fish will be when they reach the same length. (3) The change may signify recruitment of fish from another area.

SPAWNING

No detailed information on menhaden spawning areas or habits has yet appeared. Published discussions have been brief, mainly speculative, and not at all consistent, and it has been suggested that spawning occurs in bays and estuaries of the Atlantic coast, and conversely, that it takes place principally in the ocean. Kuntz and Radcliffe (1917) stated that eggs and larvae were collected in the harbor, sounds, and open ocean in the Woods Hole region. Perlmutter (1939) took eggs and larvae in Long Island waters, and Wheatland (1956) found that eggs and larvae of menhaden were widely distributed and relatively abundant in Long Island Sound.

Information on time of spawning is more precise, for reliable deductions can be made by examination of gonad condition. Thus Kuntz and Radcliffe (1917) reported spawning in New England waters in late spring or early summer, and from Chesapeake Bay southward in late fall or early winter. These authors also suggested that there may be two spawning seasons in the Chesapeake region. Scattergood *et al.* (1951) also suggested that in New England waters spawning takes place in summer. Westman and Nigrelli (1955) stated that gonads of adult fish vary considerably in "ripeness," and concluded that this was a consequence of the protracted spawning season reported by Perlmutter (1939), who found that although the peak of spawning is in May in the vicinity of Long Island, breeding occurs from May to October inclusive. Suttkus (1956) concluded that Gulf menhaden (*Brevoortia patronus*) spawn in late fall and winter in Louisiana.

Wheatland (1956) has given the most extensive information yet published on distribution of eggs and larvae, time of spawning, and oceanographic conditions that prevailed in an important menhaden fishing area. Of particular interest is the evidence that two distinct spawnings occur in Long Island Sound, in June and September. She found also that eggs taken in June were larger.

Nichols and Breder (1926) stated that menhaden reach maturity in about the third year. Scattergood *et al.* (1951) found that very few fish caught in New England waters were sexually immature, but the smallest examined by them was about 285 mm in fork length. Westman and Nigrelli (1955) concluded that all menhaden three years of age or older were adults. They concluded also that most fish reach maturity in their third year, and that a few age-group I fish caught in May 1953 seemed to be approaching maturity.

Location of spawning in Virginia

We have made no direct observations of menhaden spawning. In numerous plankton hauls in Chesapeake Bay and its estuaries at all times of year, mostly by W. H. Massmann of the Virginia Fisheries Laboratory, menhaden eggs have never been taken, although postlarvae 20 mm long or longer and juveniles are abundant in these waters at certain times. Pearson (1941) caught a limited number of eggs in late summer in plankton nets in unspecified locations in the lower part of the Bay, but concluded from the scarcity of eggs and small larvae that spawning probably occurred elsewhere. Hildebrand and Schroeder (1928) obtained no evidence to support the suggestion that there are two spawning seasons in the Chesapeake area, and believed that spawning occurred in fall.

Gonads of adult fish from purse-seine and pound-net catches showed signs of approaching maturity in late summer and fall. We have seen no fish in a fully-mature condition and we assume, as others have done, that as they approach maturity in late September, October, and November, menhaden leave the Bay to spawn elsewhere. Occasionally spent fish appeared in late fall samples also, suggesting that some spawning occurs not too far away.

Time of spawning in Virginia

As already stated, condition of gonads in commercially-caught menhaden from Chesapeake Bay points to a fall spawning, which may continue through winter, for some fish that are ripening or appear to be spent have been observed in occasional samples caught by gill net or trawls after the close

of the pound-net season. In early spring the first menhaden caught in pound nets are large, and in March these fish are obviously recently-spent. Often spent fish and occasionally ripening fish appear in pound-net catches in April. From May to the end of August or mid-September spent or almost-ripe gonads have not been observed.

Sizes and times of occurrence of larvae and juvenile menhaden in the Bay and estuaries also suggest that most spawning occurs in late fall or winter (Massmann *et al.* 1954). Postlarvae 24 to 30 mm long were collected as early as April in the Rappahannock River in 1951, and reached their greatest abundance there at the end of April and early in May. In 1957 a few postlarvae were caught by otter trawl in January. Young menhaden about 41 mm in mean length were collected in minnow seines in June and July, and larger young 94 mm in mean length were caught in September and October by surface trawling.

Evidence that two spawning peaks may occur in the Chesapeake region, one in fall and one in spring, was contained in records of lengths and weights of fish caught by pound nets. From time to time there were indications of a bimodal distribution of length and weight frequencies in age-group 0 fish. Usually these were obscured by grouping of lengths, and samples were not large enough to produce smooth distributions when original measurements in 1-mm or 1-g units were used. But these two groups showed up distinctly in combined samples for April 1955 (Fig. 1) and even more distinctly in October 1956 (Fig. 5). The two groups often were distinct in individual samples, but were lost in grouping by months. A more direct confirmation of the existence of two distinct groups of young fish is provided by the record of scale measurements, already presented in Figure 7, and mentioned also by Oglesby (1955).

Age at first spawning

Although no consistent attempt was made to classify stage of development of gonads according to a formal scale of maturity, notes were made from time to time, according to a crude classification system. Gonads small to medium in size (the testis whitish, opaque, and leaflike; the ovary faintly orange or pinkish in color, translucent, and rounded in cross-section) were called "immature." Enlarged gonads, filling at least half of the body cavity (the ovary definitely orange in color, opaque, and with visible eggs) were termed "ripening." Gonads of "spent" fish (the testis still large and firm, but with a reddish or brownish appearance, caused by the network of tiny blood vessels; the ovary flabby and shapeless, often with many small opaque eggs visible) were characterized by the presence of much vascular tissue.

In March, when the pound-net fishery began, most larger, older fish in the samples obviously were recently spent. The distinction between mature and immature fish was quite sharp: almost all fish completing their first and second years of life were immature; almost all older fish were recently spent or apparently approaching spawning. In April, many fish in their second year of life (180–220 mm long) were apparently close to spawning, and most older and larger fish seemed to be spent. Occasional ripening fish were seen in May, but by June the catch consisted almost entirely of immature fish, and gonads remained small and immature throughout summer. In September an occasional gonad showed signs of approaching maturity, and by October ripening gonads were common in age-group I and older fish. But in fall menhaden seemed to disappear from the fishery long before the spawning stage, and spent fish did not appear in the catch. Fish with spent and recovering gonads did appear in the fishery in early spring. Experience with other related species, such as Pacific herring, sardine, and anchovy, leads us to suspect that menhaden with ripening gonads in fall do not delay their spawning until spring. It seems most probable that these fish spawn shortly after they leave the fishing area, and do not return to the Bay after spawning. Large fish caught in spring almost certainly have migrated into the Bay soon after spawning, which prob-

ably takes place in the ocean not too far off the Virginia Capes.

SUMMARY AND CONCLUSIONS

Menhaden are found in Chesapeake Bay throughout the year, but their abundance and sizes vary considerably with season. In winter at least three age groups may be found: postlarvae and small young born within the previous few months; relatively small numbers of yearlings in the deeper holes in the Bay; and small numbers of two-year-olds. In early spring older fish are relatively abundant, probably part of a larger group migrating northward from waters off the South Atlantic states, but these soon decline in importance, and the dominant groups through summer and fall are fish in their first, second, and third years of life. Menhaden in their third year gradually disappear, and by winter few, if any, more than two years old are left within the Bay and estuaries. Females are slightly larger than males of the same age, but the difference is insignificant in young menhaden.

For most of the year the various age groups are not randomly distributed in the Bay and estuaries, for fish in their second year and older are found primarily in the open Bay, yearlings are taken closer to shore and in estuaries, and juveniles primarily inhabit only the estuaries, often penetrating to water that is fresh or nearly so. Within each age group also there is a segregation of sizes, the smallest fish usually inhabiting the upper parts of the rivers or inshore waters, the largest occurring downstream or offshore.

The purse-seine fishery in Virginia operates almost entirely in the open Bay and Ocean, and takes 98 per cent of the total catch. Most of the remaining catch is landed from pound nets. The purse-seine fishery extends from May to October and exploits primarily two size-groups of menhaden—in spring and early summer mostly fish in their third year of life, in late summer and fall mostly fish in their second year. The pound-net fishery also exploits primarily two size-groups, but these are one year younger—in spring and early summer mainly fish in their second year, in late summer and fall chiefly fish less than one year old.

Because menhaden are segregated so precisely according to size in Virginia waters, the first three age groups can be distinguished quite readily without reference to scales or other structures that bear a record of age. Nevertheless, it was necessary to confirm interpretations of age based on modal size-groups, and for this purpose scales were examined. An annual mark is laid down each year in spring, once the fish have developed scales, and ages based on scale reading correspond very closely with ages based on lengths. Where length-frequency distributions overlap, the scale method is necessary if maximum numbers of fish are to be aged correctly, but the method becomes progressively less reliable with older fish, because accessory growth marks and other indeterminate markings appear more frequently. For most purposes, therefore, it appears that lengths alone will give satisfactory estimates of age distribution of menhaden in the Virginia catch. Even better distinction between the first two age groups can be made on the basis of frequency distributions of weights.

The migratory nature of menhaden in Virginia, and the strong tendency to segregate and migrate according to size, make it difficult to rely on detailed accuracy of growth rates based on length or weight and age studies. Details of growth patterns inferred from migration of modal size groups or mean sizes of fish of the same age as deduced from scales, therefore, are only approximate. They show that apparent mean fork lengths at the end of each calendar year of life for menhaden caught in pound nets in Chesapeake Bay are approximately 125, 200, and 230 mm, and mean weights are about 30, 130, and 200 g. Mean lengths and weights of menhaden in the purse-seine catch are 5 to 10 per cent greater. Fish caught in fall are 10 to 20 per cent heavier than fish of the same length taken in spring.

Although menhaden probably do not spawn in Chesapeake Bay, spawning may occur not far outside the Virginia Capes. This was deduced from absence of menhaden

eggs in plankton hauls, although postlarvae and juveniles are common in the Bay and estuaries, and from absence of fully-ripe fish in commercial or experimental catches. Time of spawning in Virginia, as deduced from gonad condition, is early winter and early spring. Menhaden mature, and spawn for the first time, at about one and one-half years of age, assuming that the date of birth is January 1.

These observations, together with information gleaned from the literature, lead to the following conclusions concerning life history and migrations of the menhaden that support Virginia's fishery. Spawning occurs in late fall and early spring in the Ocean not far outside the Virginia Capes. Soon after hatching postlarvae move into the Bay and spend spring and early summer of their first year of life well up the estuaries. As they grow they move gradually downriver toward the Bay, and the larger fish appear in pound-net catches in late summer. As winter approaches, most of these young probably migrate to the Ocean and move south, returning to Chesapeake Bay and its estuaries the following spring. In spring and summer they are concentrated primarily in the lower parts of the estuaries where they are caught in some numbers in pound nets and haul seines. By fall the larger members of this group have reached the Bay, and they dominate the purse-seine catch in late summer and fall. Late in fall they leave the Bay, probably spawning outside the Capes as they move south, and the following spring the late-maturing members of this group spawn for the first time just before they enter the Bay again. In spring and early summer they form the mainstay of the purse-seine catch, but by late summer they have been superseded by the following year-class and either have been decimated by fishing, or have migrated to the Ocean in such numbers that they never again dominate or even figure prominently in the Chesapeake Bay fishery, except perhaps for a short period early in spring. Migratory habits during early life apparently are very similar to those described for croaker (*Micropogon undulatus*) by Haven (1957), and weakfish (*Cynoscion regalis*) by Massmann *et al.* (1958). Young menhaden differ in one respect from these two species in that they are pelagic, except perhaps in winter, whereas croaker and weakfish are demersal. Gunter (1957) has observed that young of these and other marine species are more likely than adults to be found in fresh or brackish waters.

The time lag between appearance in the pound-net fishery and first appearance in the infinitely more important purse-seine fishery has important implications for the menhaden industry. If prediction is of some value to plant operators, as surely it must be, they probably could estimate the success of purse-seine fishing at least a year in advance by a relatively inexpensive program designed to gather information on size and age composition and availability in the pound-net fishery. The short life of menhaden year-classes in the Virginia fishery does not imply a stable resource that will continue to produce equally bountiful catches year after year, even under the best scheme of scientific management. A single year's brood of fish plays a dominant part in the purse-seine fishery for only a year, and for this reason the fishery will be very sensitive to variations in success of spawning. Knowledge of the effects of fishing on this resource, and of the relationships between size of brood stocks and abundance of young, should be obtained as soon as possible, so that fluctuations in availability of commercial-sized fish, when they come, can be understood, and a basis for scientific management of this important fishery can be established.

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Quantitative Records of the Luminescent Flashing of Oceanic Animals at Great Depths[1]

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ABSTRACT

The bathyphotometer described previously and capable of responding to illumination as low as 10^{-7} $\mu w/cm^2$ has been modified in respect to circuit, housing, and cable, so that measurements of underwater light could be undertaken at greater depths. Graphical records were obtained of the intensity, duration, and frequency of flashes of luminescent animals at stations in the slope water SE of New York at depths as great as 3750 m (about 2⅓ miles). During the night flashes were recorded from 50 m downward and reached their greatest frequency of over 160 per minute at 100 m, with a secondary maximum of about 90 flashes per minute at 900 m. During the day flashes appeared above the background light penetrating from the surface beginning at about 400 m and increased in frequency with depth to over 110 per minute at 900 m. The brightest flashes were more than 100,000 times the dark level of the instrument. Flashes ranged from less than 0.2 sec to more than 1 sec in duration, with light from overlapping flashes lasting sometimes for more than 10 sec. Tests with animals in the laboratory indicated that the flashes recorded at sea originated within 10 m of the photometer. The nature of the vertical distribution of animal populations, as suggested by the variation in flashing, is compared with information derived from other types of observation. The brightness and varied pattern of bioluminescence is discussed in relation to the ecology of the deep sea.

INTRODUCTION

The development of the bathyphotometer containing a photomultiplier tube, described by Clarke and Wertheim (1956), made possible the extension of measurements of light in the sea to depths at which the intensity is reduced to 10^{-7} $\mu w/cm^2$ or to about 10^{-12} of full sunlight. In very clear water off the Atlantic coast, the penetration of daylight was measured to depths of about 600 m under conditions of bright sun. In the strata just above this during the middle of the day, and at all levels at night, flashes of luminescent animals were detected, and qualitative records of their occurrences were obtained (Clarke and Backus 1956). Similar luminescent flashing has been reported in Pacific waters by Boden and Kampa (1957).

Further extensions of this work immediately suggested themselves. It was desired to record quantitatively the intensity, frequency, and wave-form of the luminescent flashes that had been observed and to determine what the nature of the bioluminescence might be at still greater depths. To meet these objectives it was necessary to redesign the bathyphotometer and to provide a stronger housing with a greater length of cable. The measurements to be described extend to depths as great as 3750 m, or about 2⅓ miles, and were made in the slope water southeast of New York during the summers of 1956 and 1957.

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[1] Contribution No. 1019 from the Woods Hole Oceanographic Institution.

DESCRIPTION OF MODIFIED BATHYPHOTOMETER

Design considerations

It was desired that the instrument be capable of recording a wide range of light intensity using a fixed recorder sensitivity, and that moderate to low light levels produced by strong and weak luminescent flashes be measured with the same accuracy. These requirements imply a logarithmic-type response to light intensity that would give the same percentage accuracy at all light levels.

The photometer needed to have fast transient response in order to delineate

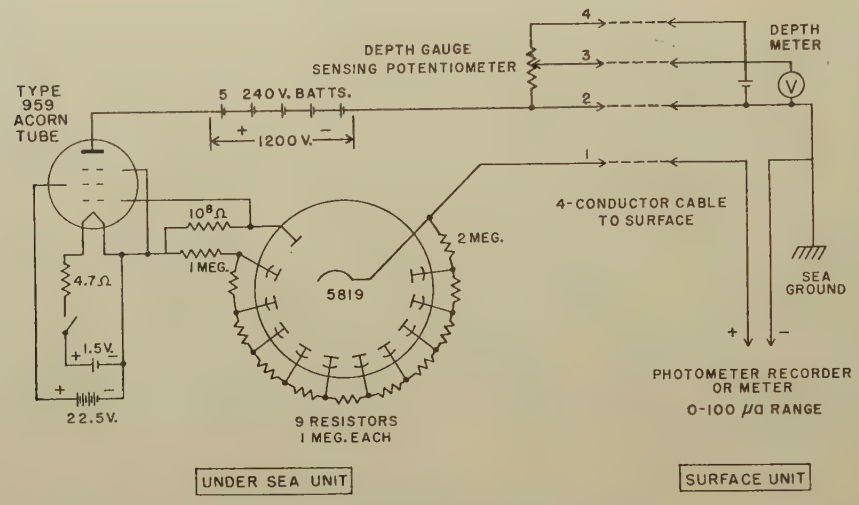

FIG. 1. Circuit for the bathyphotometer.

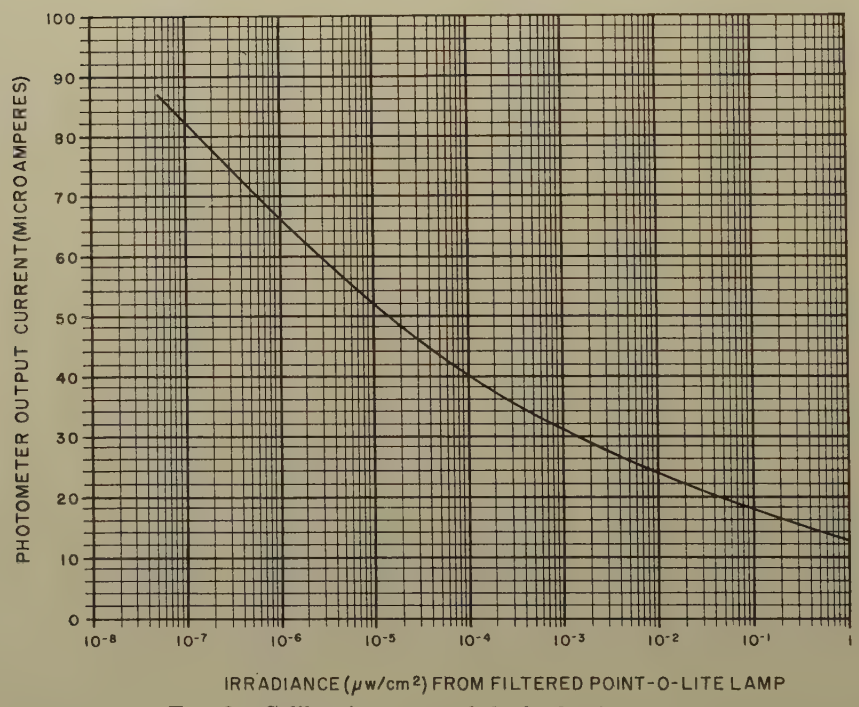

FIG. 2. Calibration curve of the bathyphotometer.

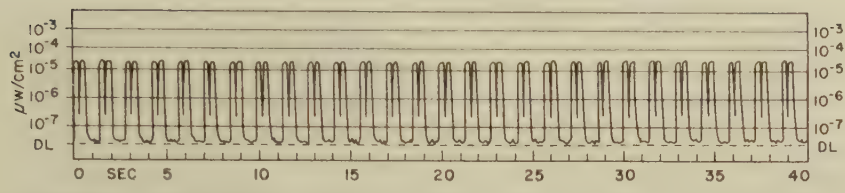

FIG. 3. Transient response test. Section of record from Sanborn Recorder during exposure of bathyphotometer to interrupted light source.

accurately the wave form of light flashes the duration of which may be of the order of 0.1 second. A similar requirement is imposed on the transient response of the recorder. A system with a rise-time much longer than 0.1 second would integrate the bioluminescent flashes, giving an erroneous value of the peak intensity.

The instrument was suspended on a long, multiconductor cable which necessarily resulted in a large electrical capacity between leads. In order to maintain the fast transient response the cable had to be terminated by a low impedance. The additional possibility of inter-conductor electrical leakage dictated that no appreciable amount of voltage, other than the photometer output signal, be present on leads in the cable. All batteries had therefore to be contained in the underwater unit.

Circuit employed

A photomultiplier tube has the property that the applied voltage necessary to maintain a fixed anode current varies logarithmically as the illumination changes. For a typical phototube (RCA type 5819), whose anode current is fixed at .01 μa, the total irradiance range of 10^{-7} to 10 $\mu w/cm^2$ implies a voltage variation of from 1150 v to 100 v. A vacuum tube in series with the high voltage supply can be used to maintain the anode current at the desired fixed value, the control grid voltage being derived from the phototube anode current by means of a high resistance in series with the anode lead. A determination of the resulting applied voltage yields the intensity of the light to be measured. Such a circuit was first used by Sweet (1946), in whose circuit the control tube was a large transmitting power tube.

The choice of the control tube to be employed in the present bathyphotometer was difficult, as the tube had to be capable of cutting off over 1,000 volts, and yet be small physically and present very little drain to the filament battery. In addition, the tube could draw only negligible grid current (less than 10^{-9} amperes) so that the grid would not divert any of the small phototube anode current. It was fortunate that a tube was found that satisfied these exceedingly stringent requirements. The type 959 "acorn" pentode was employed, which has the additional feature that the leads emerge at widely spaced points on the glass bulb, thereby reducing the problem of current leakage around the tube. Mr. J. M. Snodgrass (private communication) has independently arrived at the same choice for the control tube in a similar photometer circuit.

The complete circuit employed is shown in Figure 1. Inasmuch, as the bleeder current is uniquely related to the bleeder voltage, and hence to the light intensity, it can be passed directly to the surface via the long suspending cable. This presents a very large output impedance, eliminating any effect due to the resistance in the cable. The range of currents encountered runs from 0 to 100 μa. In the present measurements the cable was terminated at the surface by a 2,500 ohm resistor, and the voltage across this resistor was fed into a Sanborn high-speed recorder-amplifier.

The undersea circuitry was placed with the photomultiplier tube in a stainless steel cylinder one meter long with internal diameter of 7 cm and external diameter of 8.9 cm. One end cap contained a hole 5.1 cm in diameter into which the phototube fitted. The photo-emissive surface was therefore flush with the outside surface of the end cap. This was covered with a tempered glass plate 3.8 cm thick and 7.6 cm in di-

ameter. Pressure tight seals were provided by neoprene O-rings.

Calibration

Due to the geometry employed the photometer obeys a Lambert's law of collection over a solid angle of approximately π stearadians centered on the axis of the phototube. The instrument therefore measures irradiance within this angle, under the assumption that all the incident light falls in a wavelength region where the spectral response of the phototube is constant. This is approximately satisfied for the 5819 phototube by sunlight in the ocean at depths below one-hundred meters (Clarke and Wertheim 1956, Fig. 4) and is also satisfied by much of the bioluminescent emission of marine organisms thus far studied (Harvey 1952, Boden and Kampa 1957, and Kampa and Boden 1957).

The instrument was calibrated by the procedure described by Clarke and Wertheim, using a "point-O-lite" bulb in combination with Corning blue-green filter No. 4303. The incident calibration energy thus falls in the constant region of the phototube characteristic. The calibration curve is shown in Figure 2, in which the cable current is plotted against the incident energy flux. It must be stressed that this calibration is valid only for incident light that falls in the wavelength band (approx. 400–500 mμ) over which the photomultiplier tube has constant spectral sensitivity.

The transient response of the instrument was carefully measured with a pulsed light source, and was found satisfactory. The response time at the leading edge of the pulse was faster than 0.02 second; the decay time of the photometer was approximately 0.2 second at the trailing edge. A section of the record is reproduced in Figure 3. The light source was a rotary chopper with two unsymmetrically placed holes.

The pressure case also contained a depth-sensing element, consisting of a commercial pressure-actuated potentiometer (Bourns Model 304) that covered the range from 0 to 352 kg/cm^2 (5,000 lbs/in^2), equivalent to a depth in sea water of 3340 m. The potentiometer was energized by a 1.5 v battery on deck, via one lead of the suspending cable. The output voltage, uniquely related to the depth, was recorded on an adjacent channel of the Sanborn recorder.

The suspending cable used in 1956 was designated as U. S. Signal Corps "Spiral 4," and contained four insulated conductors surrounded by a steel braid, the latter furnishing a breaking strength of about 360 kg. In 1957 a change to an "Amergraph" cable, (Am. Steel & Wire Co., type 4HO) with an outside diameter of 4.8 mm and a breaking strength of 1225 kg enabled us to reach much greater depths. The cable contained four insulated copper conductors wrapped with two counterwound layers of steel armor coated with rust-resisting "Amertex." The cable was lowered from a winch equipped with slip rings, so that measurements could be performed while raising or lowering the instrument. A more complete description of the bathyphotometer as used in the present investigation is given by Hubbard (1956).

OBSERVATIONS AT SEA

Procedure

Measurements in 1956 and 1957 were made in the slope water and near the edge of the Gulf Stream at distances of 150 to 300 miles southeast of New York in the same general region as in 1955 (Fig. 4). The observations of July 17, 18, and September 6, 1956, made from the Research Vessel CARYN, were all located at the same position with a bottom depth of 2170 m. Other stations were occupied on August 11, 1957 (depth 3540 m), and on September 15, 1957 (depth 3750 m), working from the Coast Guard Cutter YAMACRAW.

In our previous observations the bathyphotometer was lowered in the upright position so that its window, provided with a diffusing disc, received light from the hemisphere above it. The transparency of the water was calculated from the intensity of light penetrating from the sea surface (Clarke and Wertheim 1956). In the study of the relation of light intensity to the vertical migration of deep-sea animals and in the previous records of luminescent flashing,

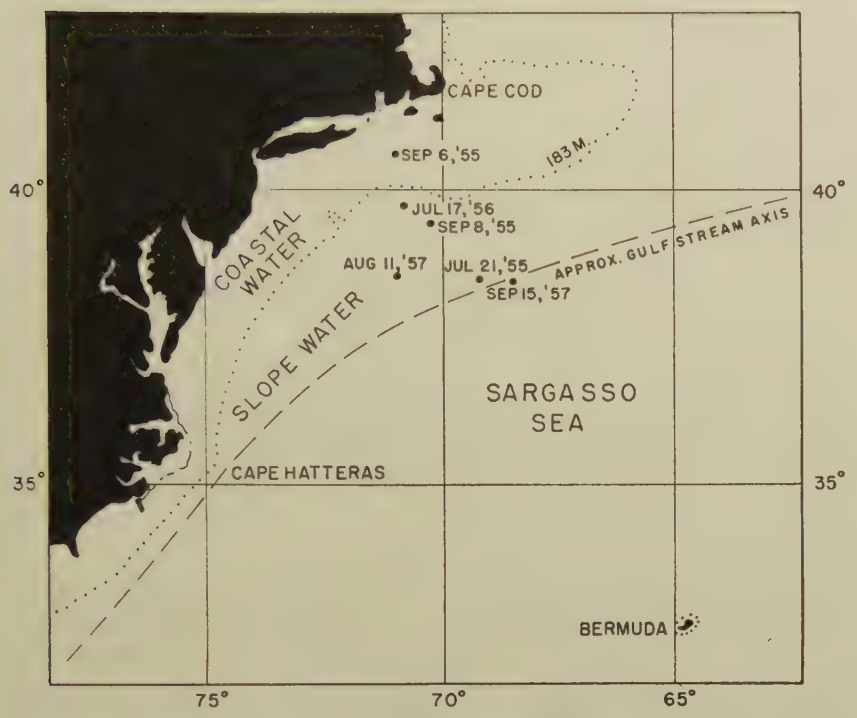

FIG. 4. Location of stations. July 17, 18, and September 6, 1956: lat 39° 39′ N, long 70° 51′ W. August 11, 1957: lat 38° 29′ N, long 71° 00′ W. September 15, 1957: lat 38° 27′ N, long 68° 33′ W.

the bathyphotometer had been employed in the same way (Clarke and Backus 1956). In the present study it was desired to measure luminescent flashes at as wide a range of depths as possible with the minimum of interference from light penetrating from the surface. For this reason the bathyphotometer was hung in the reverse position so that its clear glass window was exposed to light from below. Since in the present investigation interest was centered on the less intense portion of the light range, the protective hood and diffusing disc previously used for measurements of sunlight near the surface were not employed. The geometry of the window was such that the photometer received light from a 90° cone centered on the axis of the instrument.

For observations during the daytime the bathyphotometer, in the inverted position, was lowered to about 300 m before measurements were begun, since at shallower levels flashes would be undetectible against the background illumination from the surface. Comparison with measurements made with an upright photometer in water of similar transparency (Clarke and Wertheim 1956, Fig. 8) shows that the intensity of the upward passing light was about 1% of the downward passing light at 300 m. During the night time measurements could be made from about 50 m on down. As the bathyphotometer was lowered to greater depths, the cable winch was at first stopped for periods of several minutes while records of the illumination were made. When it was observed that the records taken while reeling in or out were not detectibly different from those taken with the winch stationary, the photometer was raised or lowered continuously at speeds up to 20 m per minute. The Sanborn recorder provided a continuous graphical record of the changes in illumination and in depth.

Day and night measurements at same station

During the daytime series of measurements of July 17, 1956, a continuous record of the light intensity from 320 m to 1,600 m was obtained. Sample sections of this

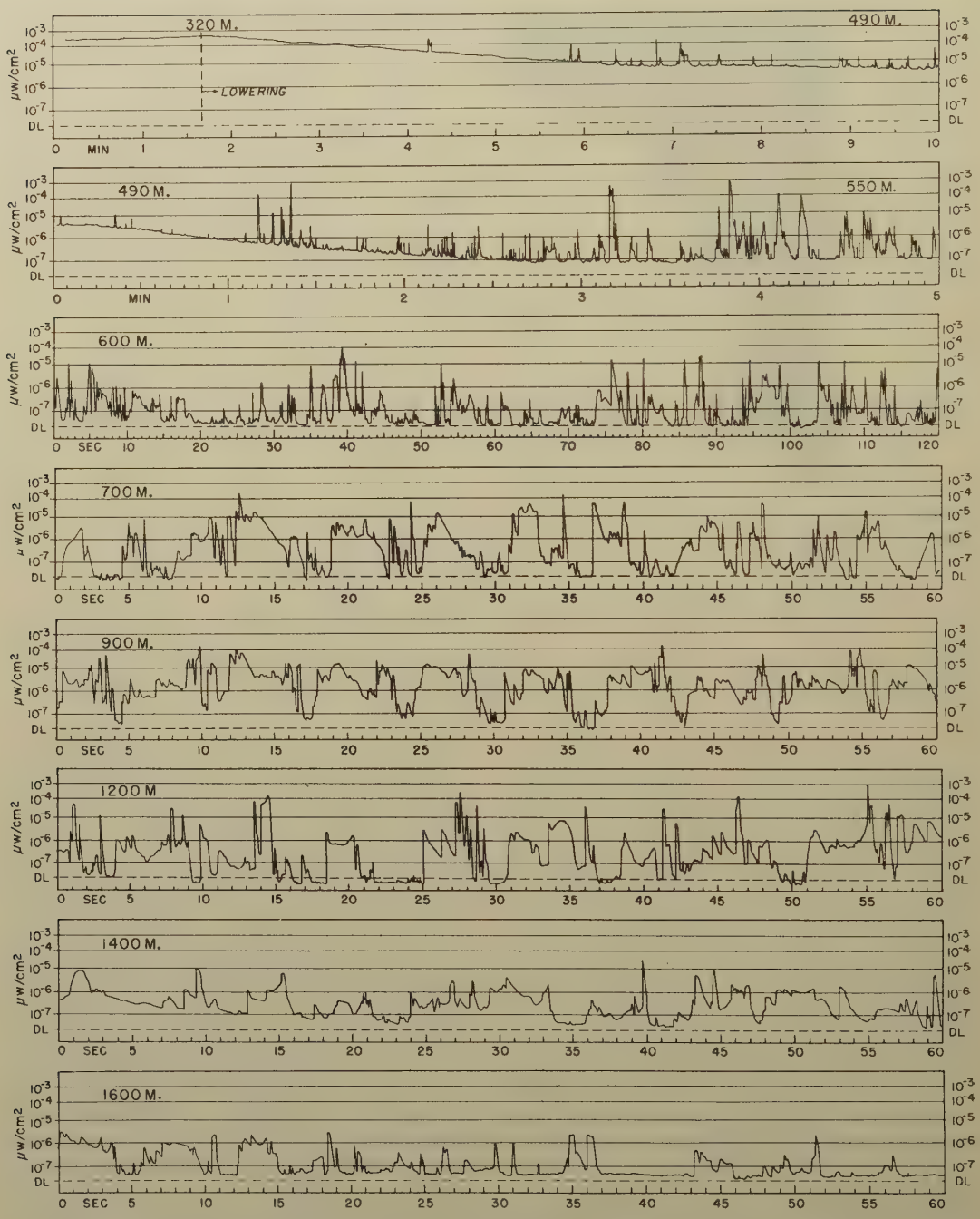

FIG. 5. Sections of record for July 17, 1956, 1008 to 1133 hrs EST showing light received on inverted photometer at indicated depths. Bottom depth 2170 m. DL = "dark level" response of the instrument.

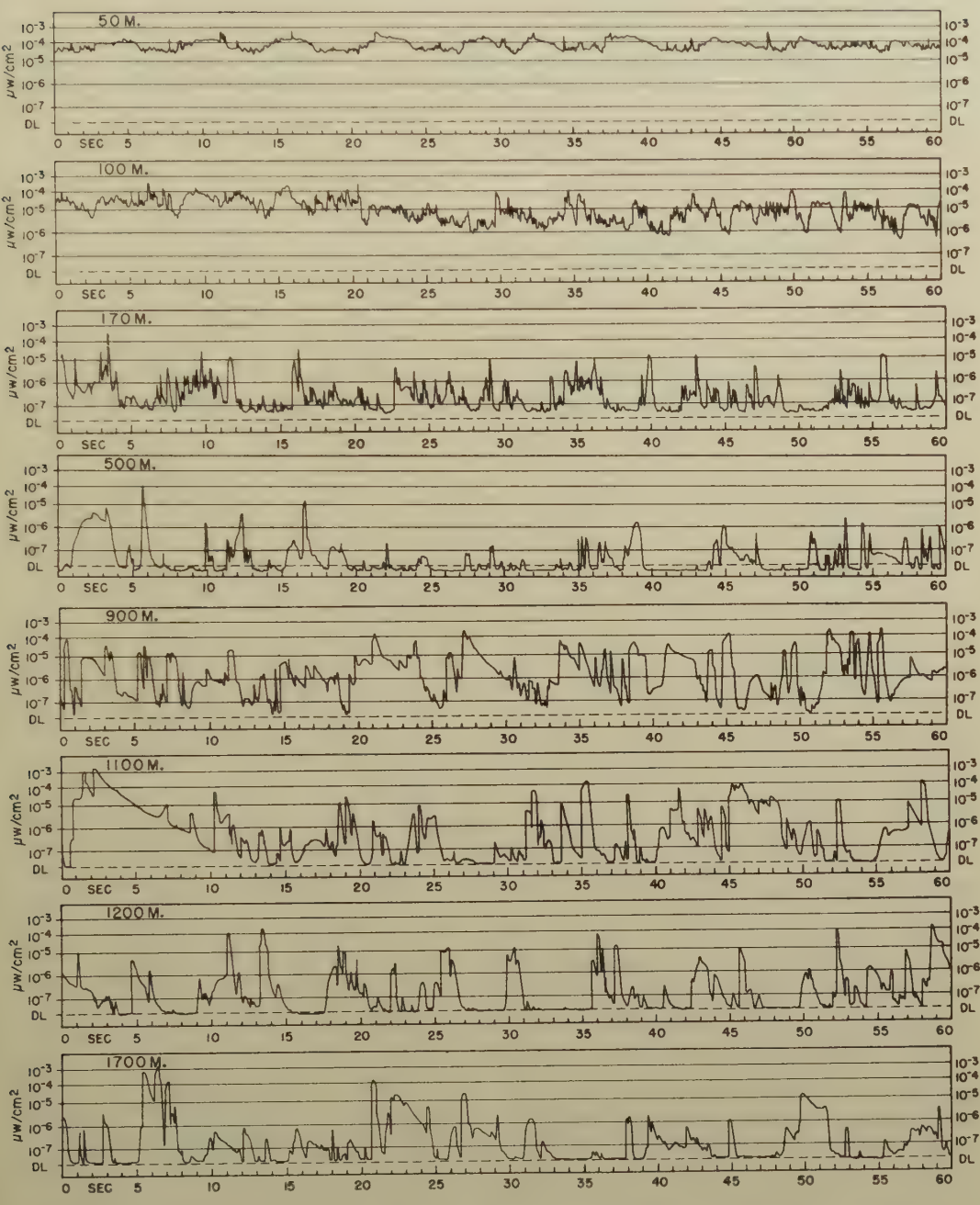

FIG. 6. Sections of record for July 18, 1956, 0000 to 0202 hrs EST. Bottom depth 2170 m.

record are reproduced photographically in Figure 5. At a depth of 320 m the intensity of the upward passing light was about 4×10^{-4} μw/cm^2 (as shown at left hand end of upper strip). As the photometer was lowered, the concomitant reduction in the intensity of the scattered light received from the surface is shown as the trace moves to the right, and in the following sections of the record. The sudden, short increases in light beginning at depths reached a few minutes after lowering began are interpreted

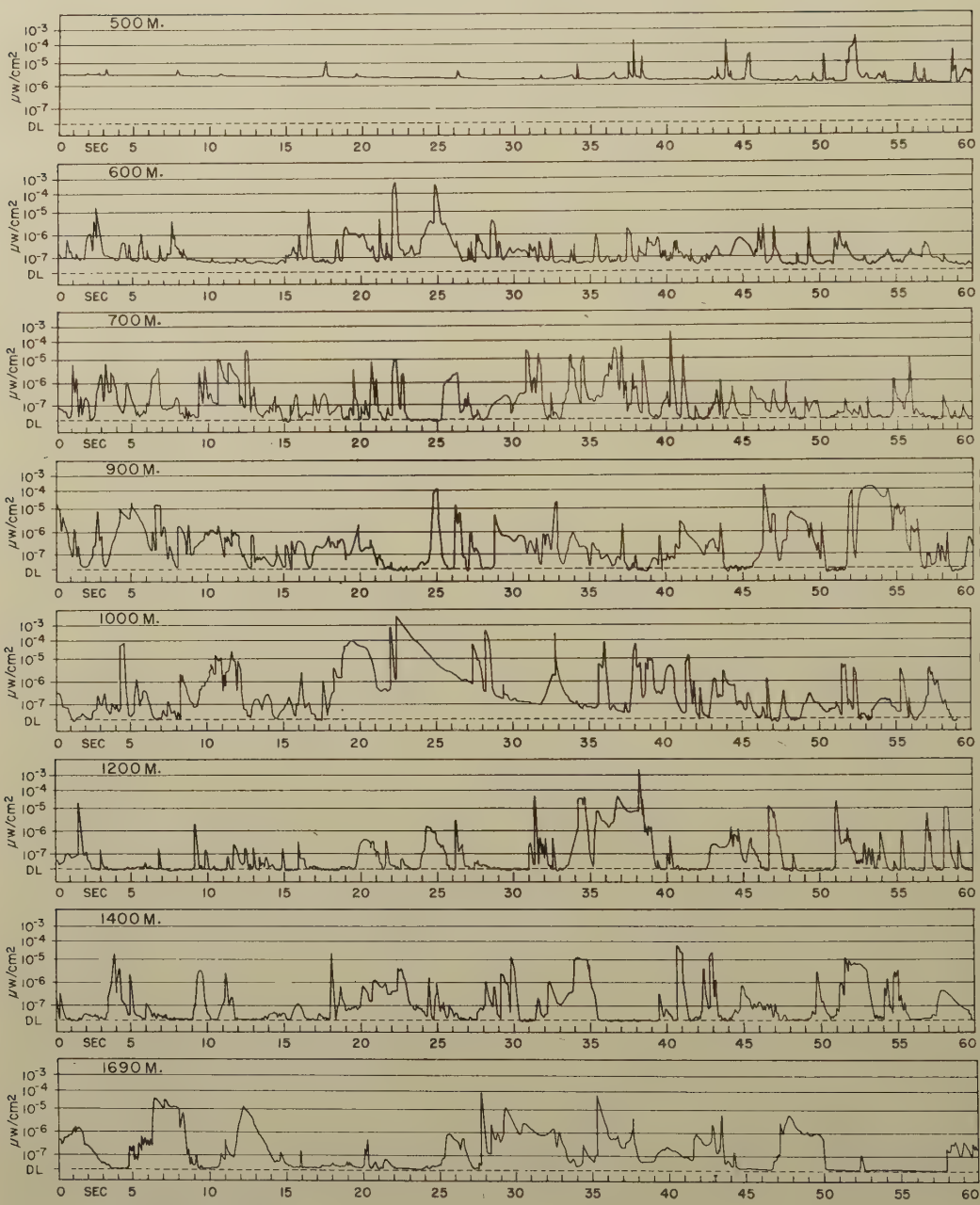

FIG. 7. Sections of record for September 6, 1956, 1000 to 1050 hrs EST. Bottom depth 2170 m.

as flashes from luminescent animals occurring at undetermined distances from the photometer within its conical field of view. At a depth of 490 m the background light had an intensity of about 4×10^{-6} μw with flashes ranging between 10^{-4} and 10^{-5} μw. As the photometer approached 500 m, a group of 4 or 5 flashes was encountered one of which reached an intensity of 10^{-3} μw. At 550 m background illumination was reduced to 10^{-7} μw, and at 620 m it had diminished to less than a fifth of this, or a value approaching the "dark level" response of the instrument and hence the

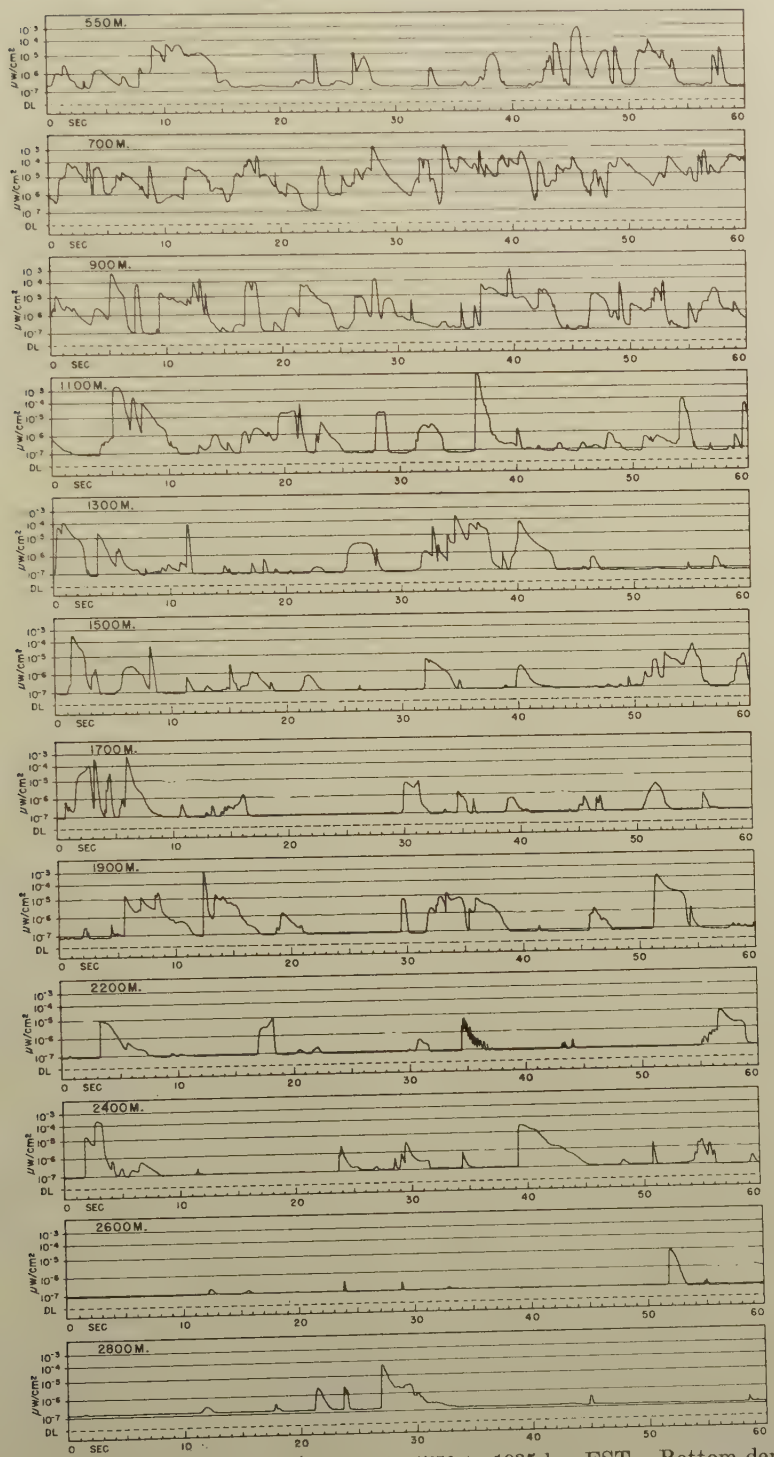

FIG. 8. Sections of record for August 11, 1957, 1750 to 1935 hrs EST. Bottom depth 3540 m.

TABLE 1. *Depth distribution of flashes in daytime*
July 17, 1956; 150 miles SE of New York

Depth (Meters)	Time EST	Number flashes per minute				Total
		10^{-2} to 10^{-4} μw/cm^2	10^{-4} to 10^{-5} μw/cm^2	10^{-5} to 10^{-6} μw/cm^2	10^{-6} to 10^{-7} μw/cm^2	
490	1008	0	7	—	—	7
550	1015	3	6	18	—	27
600	1021	1	6	21	39	67
600	1022	1	8	13	38	60
700	1028	2	13	45	36	96
800	1038	3	21	36	44	104
900	1044	3	25	73	17	118
1000	1050	4	19	41	30	94
1100	1056	3	14	46	33	96
1200	1103	4	15	28	37	80
1300	1108	2	21	41	21	85
1400	1117	0	3	24	39	66
1500	1121	0	0	21	42	63
1600	1133	0	0	16	38	54

approximate lower limit of measurement. As the background illumination fell below 10^{-5} μw, and later below 10^{-6} μw, an increasing number of flashes appeared on the record. Flashes were found in abundance at every level from their first appearance to the maximum depth attained. A scrutiny of the entire record, of which the excerpts reproduced here are only a small part, reveals the fact that at no level below 500 m were there periods of more than a few seconds without flashing.

Changes in the frequency, intensity, and duration of the flashes at the various levels may be observed from the graphical record and also from Table 1 in which actual counts of the number of flashes per minute for four magnitudes of intensity are presented. The brightest flashes—between 10^{-3} and 10^{-4} μw in intensity—occurred between 1 and 4 times per minute at all levels except at 490 m and below 1,300 m. Flashes reaching the next lower order of magnitude were more frequent, and flashes less intense than 10^{-5} μw occurred still more abundantly. The total number of flashes of all magnitudes reached a maximum of 118 per minute at 900 m and dropped off to 54 per minute at 1,600 m. There was a noticeable lack of larger flashes at 1,400 m and below, with longer intervals between flashes. The duration of flashes varied from about 0.2 sec, which is the shortest which could be reliably traced by the Sanborn recorder, to single flashes or combinations of overlapping flashes, lasting for five seconds or more. The apparent rhythm in the flashing with a period of about six seconds, seen in certain parts of the record, is probably due to the roll of the ship. The resulting periodic movement of the supporting cable may have produced turbulence or caused the photometer to strike luminescent animals near the window of the instrument and thus stimulated them to give extra or prolonged flashes. It might be argued that all or most of the flashes recorded were the result of mechanical stimulation by the moving photometer. However, the fact that sections of the record with no apparent periodicity show many flashes indicates that at least much of the flashing is spontaneously induced by the animals themselves.

During the night following the foregoing series of measurements and at the same station a continuous record of light was made from 50 m to 1,700 m. Sample sections obtained between 0000 and 0200 hrs on July 18, 1956, are presented in Figure 6, and a summary of the frequency distribution of flash size at representative depths is given in Table 2. The background intensity of scattered light received by the inverted photometer at a depth of 50 m at midnight with no moon and partly cloudy sky was about 8×10^{-5} μw. Many flashes slightly brighter than this were recorded together with a periodic oscillation of the background or of the intensity of flashing. This rhythm, with a period of about 6 sec, was again probably due to the roll of the ship causing a mechanical stimulation of the luminescent animals or possibly a slight tilting of the photometer which might thus have received more light from the sky. The record shows much less indication of periodicity at greater depths. Because of the fluctuating level of background light no precise count of the number of flashes at 50 m can be made especially for intensities lower than 10^{-4} μw. However, no reason suggests itself to suppose that the flashes in the record rising to intensities above 10^{-4}

TABLE 2. *Depth distribution of flashes at night*

July 18, 1956; 150 miles SE of New York

Depth (Meters)	Time (EST)	Number of flashes per minute				Total
		10^{-3} to 10^{-4} $\mu w/cm^2$	10^{-4} to 10^{-5} $\mu w/cm^2$	10^{-5} to 10^{-6} $\mu w/cm^2$	10^{-6} to 10^{-7} $\mu w/cm^2$	
50	0000	23	—	—	—	23
100	0002	6	88	72	—	166
170	0004	1	11	45	78	135
330	0100	1	3	8	24	36
400	0103	0	1	12	20	33
500	0108	1	2	9	29	41
600	0111	0	5	8	35	48
700	0115	1	3	16	40	60
800	0120	4	16	32	34	86
900	0124	8	27	33	25	93
1000	0127	6	19	28	39	92
1100	0131	4	16	23	27	70
1200	0138	0	4	10	20	34
1300	0148	1	5	10	35	51
1400	0151	0	6	19	38	63
1500	0154	0	6	13	29	48
1600	0159	3	5	14	26	48
1700	0202	4	4	15	37	60

μw were produced by anything other than the flashes of animals. The number of flashes of this magnitude is much greater than at any other level and thus indicates at this depth that either the number of animals producing the brightest flashes was larger or that the animals present flashed more frequently.

At 100 m the total of 166 flashes recorded in one minute was the largest number observed at any level. Of these the flashes between 10^{-4} and 10^{-5} μw were the most numerous, but almost as many ranged between 10^{-5} and 10^{-6} μw. At 170 m the background light had dropped below 10^{-7} μw. Of the 135 flashes observed in one minute the majority ranged between 10^{-6} and 10^{-7} μw. From this depth downward the largest number of flashes at each level (with one exception) were in this weakest intensity category, with smaller numbers in successively brighter ranges of intensity. Below 170 m the total number of flashes per minute dropped off sharply and then increased to a secondary maximum at 900 m with considerably lower numbers from this level down to 1,700 m.

From 600 m down at each level the total number of flashes was less at night than during the day, suggesting that many luminescent animals may have migrated from depths below 600 m to the upper levels during the evening. Presumably a large number of the animals producing such an abundance of flashing at 100 m and at 170 m migrated to levels deeper than 500 m during the day, but it is possible that many of them remained in the upper layers. If they continued to flash during the day, their luminescence could not have been detected because of the high level of the ambient light. More probably the flashing of any animals near the surface would be inhibited by the high illumination.

At 500 m and below many periods of prolonged luminescence were recorded due either to sustained light emission from one animal or to the overlapping of flashes of several animals. In the first part of the record at 1,100 m the emitted light exceeded 10^{-3} μw, or almost 100,000 times the intensity represented by the dark level, and the light remained above the baseline for more than 13 seconds. At 900 m the light was almost continuously above the dark level, with the illumination dropping below 10^{-7} μw only occasionally and usually for less than a second. This situation may be contrasted to the conditions at 500 m and 1,100 m. At no depth did the illumination remain at or below the dark level for more than four or five seconds at a time; periods of one to four seconds without flashes occurred at 500 m but chiefly at depths below 900 m.

A third series of measurements, made at the same station on September 6, 1956, extended from 500 m to 1,690 m, and sections of the record are reproduced in Figure 7. The illumination received by the inverted photometer at 500 m was about 3×10^{-6} μw, or about the same intensity as reported for that depth during the measurements of July 17. Flashes above the ambient light level were encountered at strata just below 500 m and became increasingly abundant at depths greater than 600 m. A maximum in the frequency of flashing was again encountered at 900 m.

Deeper observations

The modifications of the photometer described above made possible the exploration of bioluminescent flashing at still greater depths in 1957. Representative sections of the record from 550 to 2800 m made in the late afternoon of August 11 are presented in Figure 8. During this series the dark level of the instrument was somewhat higher than for previous measurements and occurred at about 10^{-7} $\mu w/cm^2$. At 550 m a moderate amount of bioluminescent activity was recorded but with intervals of 5 sec or so with little or no flashing. A maximum of luminescence was encountered between 700 and 900 m, and in this stratum the overlapping flashes maintained the illumination well above the dark level for more than a half a minute at a time. At greater depths flashing was less frequent, but at least a few flashes of high intensity were found at every level down to 2000 m. Below this depth luminescent activity became progressively curtailed with periods of more than 20 sec during which no flash was recorded. However, a few flashes with intensities greater than 10^{-5} $\mu w/cm^2$ were observed at 2800 m, or about 1.7 miles beneath the surface. At 2200 m a curious pattern of luminescent discharge was recorded in which the light rose abruptly to an intensity of about 100 times the dark level and then dropped off in a cascade of peaks of decreasing size. Future investigation may show that certain types of animals can be identified by the pattern of their flashing.

An even deeper series of measurements was carried out with the same instrument by Mr. Lloyd Breslau during the night of Sept. 15–16, 1957. Excerpts of the record (Fig. 9) give a synoptic view of the light conditions for the entire depth from 100 to 3750 m, or about 2.3 miles. The record shows a profound variation in the frequency, intensity, and duration of flashing at various levels. Primary and secondary maxima in luminescent activity occurred at 200 m and 900 m in similar fashion to previous night records. At a depth of 100 m the general level of background light was about 10 times the dark level of the instrument. Flashing was frequent, but the majority of flashes were weaker than 10^{-5} μw. At 200 m more flashes were recorded, with a considerable number brighter than 10^{-4} μw and a few brighter than 10^{-3} μw. The frequency of flashing was so high that the light did not remain steady for more than half a second at a time. The number of flashing animals was very much less at 300 m and 500 m, but the flashing rate began to increase again at 600 m, reached a peak at 900 m, and continued high to about 1000 m. Although the flashes were fewer at this depth than at 200 m, they were more sustained, so that the illumination was well above the dark level of the instrument for periods of ten seconds or more. Below 1300 m luminescence gradually diminished, but many of the individual discharges were as intense as at shallower depths. At 2700 m a few bright flashes occurred within each one-minute period. At greater depths, however, the flashes were weaker and fewer than one per minute. As the photometer approached the bottom at 3750 m, there was no indication of an increase in the amount in luminescent activity.

Laboratory observations

In the foregoing observations at sea the flashes recorded may have been produced by animals of either large or small size at a variety of distances from the receiving window of the photometer. In order to ascertain the appearance on our record of the trace of a known animal's flash at a known distance, recordings were made in the laboratory of luminescent discharges of the ctenophore, *Mnemiopsis leidyi*, which occurs in Woods Hole waters during August, and of the common firefly, *Photuris pennsylvanica*.

One specimen of *Mnemiopsis* was placed in a square-sided glass vessel of sea water and set in a light-tight black box into one end of which the head of the photometer was inserted in a fashion similar to that described by Clarke and Backus (1956). A record of flashes, sections of which are shown in Figure 10, was made with the same photometer sensitivity and the same speed of recording as in the observations at sea.

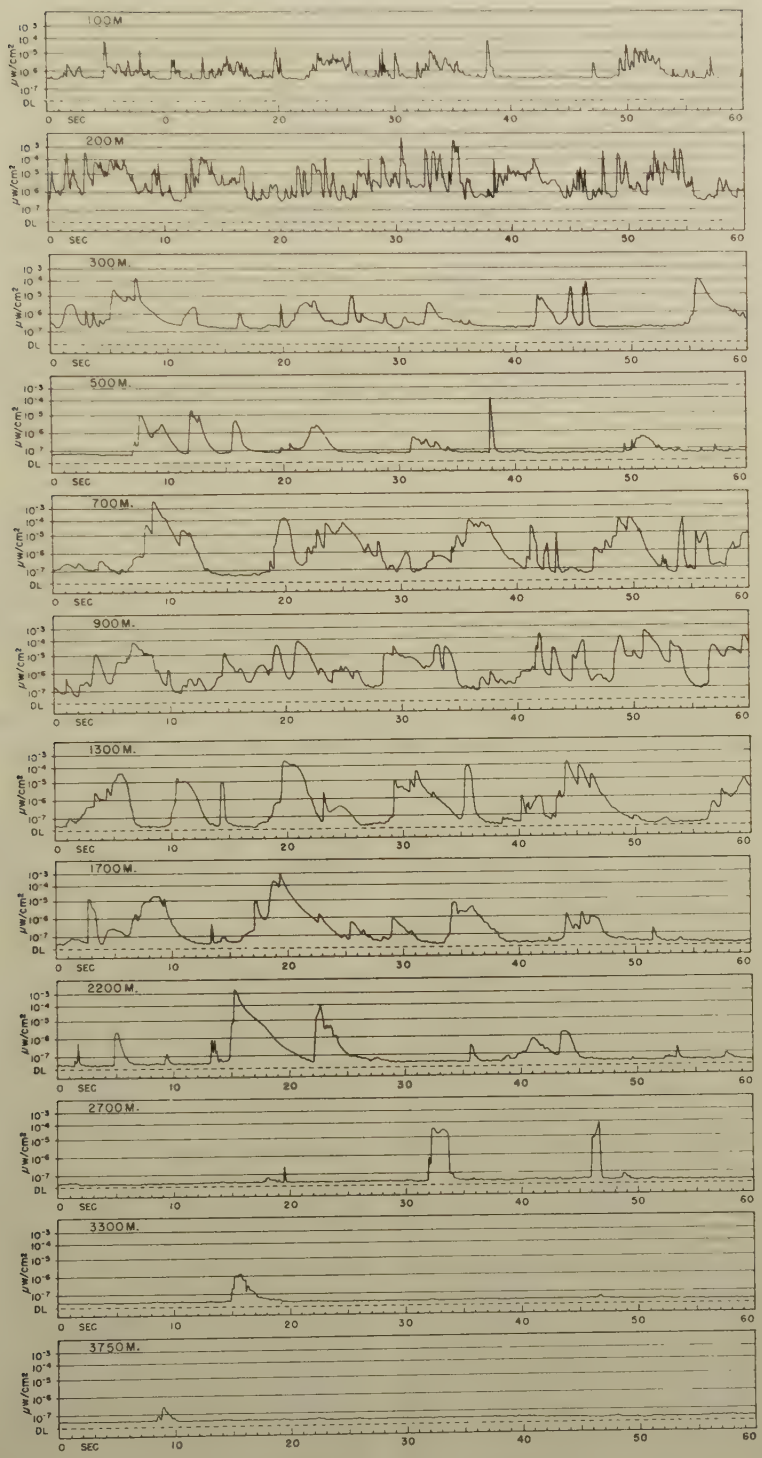

FIG. 9. Sections of record for September 15–16, 1957, 2300 to 0100 hrs EST. Bottom depth 3750 m.

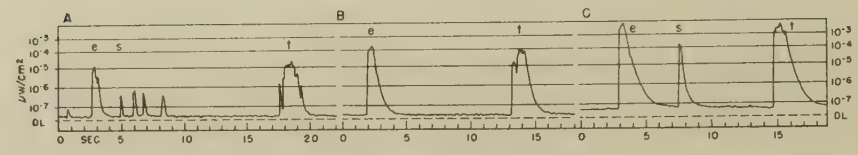

FIG. 10. Flashes produced by a 55-mm specimen of ctenophore, *Mnemiopsis leidyi*, in the laboratory on August 1, 1957, at distances from the photometer of (A) 80 cm, (B) 37 cm, and (C) 1 cm. Luminescence resulted from (*e*) electrical stimulation, (*t*) mechanical stimulation, and (*s*) spontaneous activity.

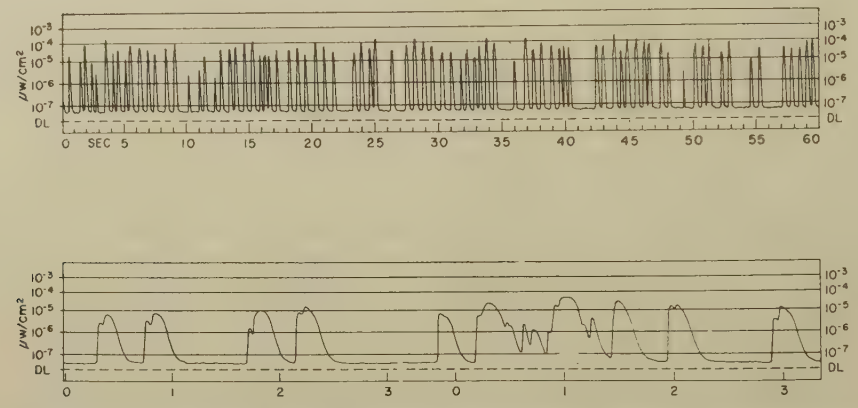

FIG. 11. Spontaneous flashes of firefly, *Photuris pennsylvanica* recorded in the laboratory on July 17, 1957, at 37 cm from photometer at regular speed (upper) and at increased speed (lower) of the recorder.

Tracings were obtained of light flashes with the animal at distances of 80 cm, 37 cm, and 1 cm from the photometer during spontaneous luminescence as well as following electrical and mechanical stimulation. The discharges were greater following artificial stimulation; in some instances the response was unimodal and in some it was irregular. Generally, the light flash reached its peak of intensity within a small fraction of a second and then died away over the ensuing few seconds. The form of light discharge is thus similar to many records obtained at sea.

The firefly was placed in a glass vial and held by a plug of cotton against the glass with its ventral surface toward the photometer at a distance of 37 cm. The flashing was spontaneous and fairly regular with an average period of slightly less than one second (82 flashes per minute) although there were some longer and shorter intervals. Sections of the record at the previously used speed, and at a much higher speed, are presented in Figure 11. The bimodal nature of many of the flashes is probably due to a slight overlap in the discharge of the two photophores of the firefly.

DISCUSSION

The foregoing measurements together with those of Boden and Kampa (1957) and Kampa and Boden (1957) represent a first step in the quantitative investigation of luminescence in the deep sea. The present records reveal the nature of the changes in frequency, intensity, and duration of flashing under the circumstances of observation at the depths and locations sampled. It is obvious that luminescence may differ greatly from place to place, and more information is desired about certain aspects of the measurements before general conclusions can be reached. For example, with the existing arrangement of the apparatus neither the exact position of the flashing animal relative to the photometer nor the extent to which mechanical agitation may affect the amount of luminescence is known.

Observations in a variety of other localities and further refinements of the procedure are planned.

As an additional extension of the investigation an instrument known as the luminescence camera has been designed and constructed by Breslau and Edgerton (1958, 1959) for the purpose of identifying the principal flashing animals in various strata by causing them to take their own photographs. The instrument consists of a photomultiplier detector arranged to pick up light from a cone of water outside its receiving window, an electronic flash which, when discharged, illuminates this cone, and a camera in a water-tight case which is focused upon the same volume of water. When any luminescent animal moves into the sensitive cone, its flash triggers the device with the result that the electronic flash is set off, and the camera takes a picture of the animal. The device is designed to operate at depths down to 6000 m, and it may be used in conjunction with the bathyphotometer. Photographs already obtained in the upper layers indicate that a large number of flashers are extremely small animals, although many euphausiids, ctenophores, medusae, and siphonophores have been photographed. The camera is now being employed at greater depths, and the results will be reported as soon as opportunity permits.

Certain information in regard to the effect of the distance of the animals from the photometer may be deduced from the laboratory experiments. The electrical stimulation of *Mnemiopsis* in the laboratory produced an illumination of about 10^{-5} $\mu w/cm^2$ at the photometer window at a distance of 80 cm; at 37 cm the flash was 10x brighter, and at 1 cm it was about 700x brighter than at 80 cm. If the animal's luminous discharge is treated as a point source and the inverse square law applied, we find that at 800 cm the light intensity would have been reduced to 1 per cent of its value at 80 cm, or to a value approximately 10x the dark level of the instrument. At sea the light emitted would be further reduced by adsorption and scattering by the water through which it passed, but the additional reduction would amount to only about 40 per cent for passage through 8 m of clear ocean water. The measured intensity of flashes received from points not on the axis of the instrument would be reduced by $\cos^2 \theta$, but for the extreme measurable angle of 45° the reduction would be only one half. We may conclude, therefore, that luminescent flashes of any animal equal in magnitude to the largest recorded in the laboratory for *Mnemiopsis* could have been detected by the bathyphotometer in the sea at distances up to 8 m or slightly more.

Very little is known about the absolute magnitude of the luminescent flashes of oceanic animals under natural conditions, and relatively few laboratory studies have been conducted (Harvey 1952). It seems probable, however, that few deep sea animals produce flashes that are very much brighter than *Mnemiopsis*. If this be true, we may conclude that the bathyphotometer does not record flashes to any important extent from animals farther away than 10 m, and that it does provide a reliable index of the population of the brightest flashers within this distance. The smallest signals on the tracing may be due to flashes from the brightest animals at extreme range or at oblique angles, or to weak flashes near the photometer window. During laboratory observations when water from Woods Hole harbor was placed in a small, square-sided glass vessel close to the window of the bathyphotometer, individual flashes of protozoans such as *Tintinnopsis* were recorded. Thus certain of the light impulses detected in the sea may be from relatively minute animals which have flashed spontaneously or which have been stimulated by striking the photometer window. On any occasion when large numbers of small luminescent forms are present, a strong movement of the photometer, due for example to the roll of the ship, would be expected to result in a volley of discharges. Many flashes may overlap to produce a seemingly continuous glow, such as the familiar "burning water" seen at the sea surface due to breaking waves or to an object moving through the water.

The differences in the frequency and

nature of light flashes at different depths may be considered in relation to the vertical distribution of the pelagic fauna. General summaries of the changes in the kinds and abundance of animals with increasing depths in the open sea are presented by Marshall (1954) and Hardy (1956). Direct observations from the bathyscaph in the Mediterranean and off the Atlantic coast have been reported by Bernard (1955), Furnestin (1955), Trégouboff (1956), Pérès and Picard (1956), Pérès, Picard, and Ruivo (1957), Piccard and Dietz (1957), and Dietz (1959). These investigators found distinct strata of abundance and of scarcity at various levels from the surface to depths of several thousand meters. For the most part their observations in deep water were made with the underwater search lights of the bathyscaph turned on but occasional periods with the lights off provided some information on the occurrence of bioluminescence. Off the coast of Portugal Pérès, Picard, and Ruivo (1957) observed that the plankton which was rich near the surface diminished to a minimum at about 300–400 m. From 400 m to 800 m the plankton again became rather rich and then diminished to another minimum between 1,100 and 1,400 m. Piccard and Dietz (1957) state: "Particularly during the 1956 dives to 1100, 2000, and 3700 m the bathyscaph traversed zones between 500 to 800 m where the density of life appeared to be remarkable. Hundreds of tunicates could be seen at one time, mainly salpa (½ – 1 cm long) which flashed with an almost white luminescence. At times there seemed to be about ten dozen salpa per cubic meter of water.... With the lights extinguished in deep water a small point of bioluminescence is seen every few seconds."

Several series of quantitative plankton hauls were made by Leavitt (1938) in the deep water southeast of New York, in the same general region as the present investigation. Using closing nets towed at levels 200 m apart from 200 m to 3000 m. Leavitt found major peaks in the size of the total catches at depths of 200 m and 800 m and a minor peak at 1600 m. At his Station 2216, located 110 miles SE of our July 17–18, 1957 station, the percentages of fish, copepods, euphausiids, decapods, chaetognaths, salps, and coelenterates taken in each stratum were determined. Each of these taxonomic groups showed a sharp maximum at 800 m and some showed secondary peaks of abundance at 200 m and 1600 m. Numbers were very small for all groups at 400 m, from 1000 to 1400 m, and below 2000 m. It is of great interest to note that a general correspondence exists between the pattern of the vertical distribution of all animals taken in Leavitt's plankton nets and our records of the vertical changes in luminescence, even though at least some of the animals caught in the earlier study were presumably not luminescent. Since Leavitt did not sample the 900 m level, his maximum at 800 m may very likely represent the same population as that which produced the peak at 900 m in our records of light flashes. Our maximum at 900 m occurred during both day and night observations; our maximum in the upper 100 or 200 m could be observed only during the night.

None of the maxima in abundance discussed above correspond with the level of the deep scattering layers in this region as detected by the usual echo-sounder recorders. During the previous year's investigation a single scattering layer was found at about 400 m during the middle of the day, and one of two scattering layers was followed up to about 50 m in the evening after which echoes from it merged with the near-surface reverberation (Clarke and Backus 1956). During the day and during twilight hours the scattering layers were found at levels where the ambient daylight would obscure the weaker flashes, and in the present study no increase in the abundance of flashes brighter than the background was detected in the water strata occupied by the scattering layer animals.

Any correspondence between the total abundance of pelagic animals, the amount of luminescence, and the occurrence of sound scatterers is of interest, but instances of non-conformity are not to be regarded as surprising. Large aggregations of animals may exist without luminescent

forms necessarily being among them. A strong sonic scattering layer may be produced by a group of animals of a type that reflect sound effectively but which are not as numerous as animals at another depth which are poor sound scatterers. However, light is probably so important in controlling the vertical distribution and the activity of many, if not most, pelagic animals that a definite relation between the illumination from the surface, the abundance of animals, and the occurrence of luminescence might be expected. The present measurements indicate a significant increase in luminescent flashing in the neighborhood of 900 m. This is probably just below the depth at which light from the surface affects animal activity.

The minimum illumination in the central part of the visible spectrum under which shallow-water fishes can see small objects is probably 10^{-10} of sunlight (Clarke 1936). The human eye has approximately the same threshold. If deep-sea fishes can see equally well in the blue region of the spectrum, minimum light for their vision would exist at a depth of about 600 m in water as transparent as this part of the ocean (Clarke and Wertheim 1956). Observers in the bathyscaph could detect daylight when looking horizontally and could see light reflected from objects in the water at depths down to about 600 m (Dietz 1959). If a deep-sea fish had eyes 100 times more sensitive than man's, it could see objects 120 m deeper, or at a total depth of about 720 m, since daylight is reduced by a factor of 10 for each depth increase of 60 m in clear ocean water (Clarke and Wertheim 1956). Animals looking toward the surface could presumably detect the difference between day and night at somewhat greater depths, provided that bioluminescence did not interfere. The studies of Denton and Warren (1957) indicate that the eyes of certain deep-sea fishes are 60–120 times more sensitive to a large field of daylight than the human eye. In any situation in which bioluminescence is as strong as that received by our photometer at every depth investigated, the visual detection of small objects illuminated by biological light would be easily possible. In the water layers above the ultimate maximum for daylight detection, deep-sea animals can presumably perceive differences in light received from the surface during periods of no nearby luminescent discharge, and perhaps can distinguish qualitatively at all times between background daylight and bioluminescence from localized sources.

Certain animals have been found to carry out diurnal vertical migrations, presumably in response to changes in submarine daylight, at depths of 800 m or possibly slightly greater (Waterman *et al.* 1939). A depth of about 900 m to 1000 m would appear to be the shallowest level in clear water at which day and night changes in illumination would be below the threshold of perception and at which daylight would never be sufficiently strong for the inhabitants to be seen by their predators or by their prey. For these or related reasons a large population may have come to occupy this stratum. The fact that a high degree of luminescence has been observed to characterize the animals at this depth might appear to be contradictory to the foregoing suggestion, but the flashing might have developed as counter-measures of defense, recognition, or the luring of prey. The findings of other investigators, cited above, suggest that a concentration of pelagic animals at about 900 m may be of widespread occurrence.

Whether or not any validity will be found in the foregoing speculations, the present investigation has shown that bioluminescence may provide detectible illumination at depths at least as great as 3750 m. Although further exploration will be required before generalities can be established, the variation in frequency, intensity, and duration of the flashes here reported indicate the presence of very different populations at various depths. The brightness and varied pattern of the bioluminescence observed suggest that the phenomenon is probably of even greater significance in the ecology of the deep sea than previously supposed.

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The Effect of Grain Size on the Distribution of Small Invertebrates Inhabiting the Beaches of Puget Sound

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ABSTRACT

The position with respect to the general configuration of the shore-line and the direction and force of the currents of five localities in Puget Sound is such that they represent a series of decreasing exposure. Richmond Beach being the most exposed, Bainbridge Island the most sheltered locality. This is reflected in the composition of the substrate, the former locality having the coarsest, the latter the finest substrate. Perpendicular to this horizontal gradient there runs a vertical gradient due to the tide, the coarse grades being deposited in the upper, the fine grades in the lower intertidal.

The intertidal distribution of at least some of the more common species of invertebrates on the beaches is not so much determined by levels of tidal water as by the pattern of distribution of certain grades of substrate. For example, species of this sort will penetrate into the upper intertidal if the preferred substrate occurs there, but they will remain in the lower intertidal if the substrate is confined to this zone.

On the beaches there exist mixtures of sand which constitute barriers separating major faunal components from each other. It is assumed that substrates with a median diameter of approximately 200μ constitute such a barrier separating the bulk of interstitial sliders from a great number of burrowing animals. The former can move only in sand coarse enough to maintain an interstitial system; the latter, for mechanical reasons, will find fine sand more favorable than coarse. This distinction, however, does not apply to nematodes which are able to move even in the interstices of fine sand. There are various ways in which grain size and shape can influence the distribution of the fauna. These types of relationship are discussed.

INTRODUCTION

The small invertebrates living on marine beaches have only within the last 25 years become the subject of thorough studies. Although a number of excellent papers dealing with particular groups have recently appeared, stimulated to a large extent by the work of Remane (1933), *e.g.* Ax (1951), Gerlach (1953), more general papers treating the whole fauna from a predominantly ecological point of view are still scarce, the greatest bulk of information on that subject being found in Remane (1940), Pearse *et al.* (1942), several of the papers by Delamare Debouteville and his associates (*e.g.* 1955), and in the review by Pennak (1950). It is particularly the effect of single environmental factors on the composition and pecularities of the sand fauna which has yet to be analyzed in more detail. Since the most important factor in this connection is the substrate itself, more information on the relationship between the latter and the fauna in general would be desirable.

The present paper is a contribution to this topic. The work on which it is based was undertaken between October 1955 and August 1956 when the author held a research fellowship of the International Cooperation Administration at the Department of Zoology, University of Washington, Seattle.

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Mrs. G. C. Carl (J. F. L. Hart), Victoria (cumaceans); Dr. P. A. Chappuis, Toulouse (copepods); Dr. Olga Hartman, Los Angeles (polychaetes); Mr. Clarence R. Shoemaker, Washington, D. C. (amphipods); Dr. Willis L. Tressler, Washington, D. C. (ostracods). Dr. Melville Hatch, Seattle, helped with the other peracarids.

The following groups were studied by

myself: nematodes, gastrotrichs, archiannelids, and the single hydrozoan (Wieser 1957a, b, 1958, 1959).

My ambition was to cover all the small invertebrates occurring on the beaches, but unfortunately for one of the most important groups, the turbellarians, no specialist could be found. Otherwise all numerically important groups are included in my investigation. Of the less abundant taxa the small lamellibranchs had to be excluded.

A general account of the oceanographic features of the area under investigation can be found in University of Washington (1953), Wang (1955), and in my paper on the Puget Sound nematodes (Wieser 1959).

All the indications of tidal height in the following pages are referred to mean lower low and are given in feet.

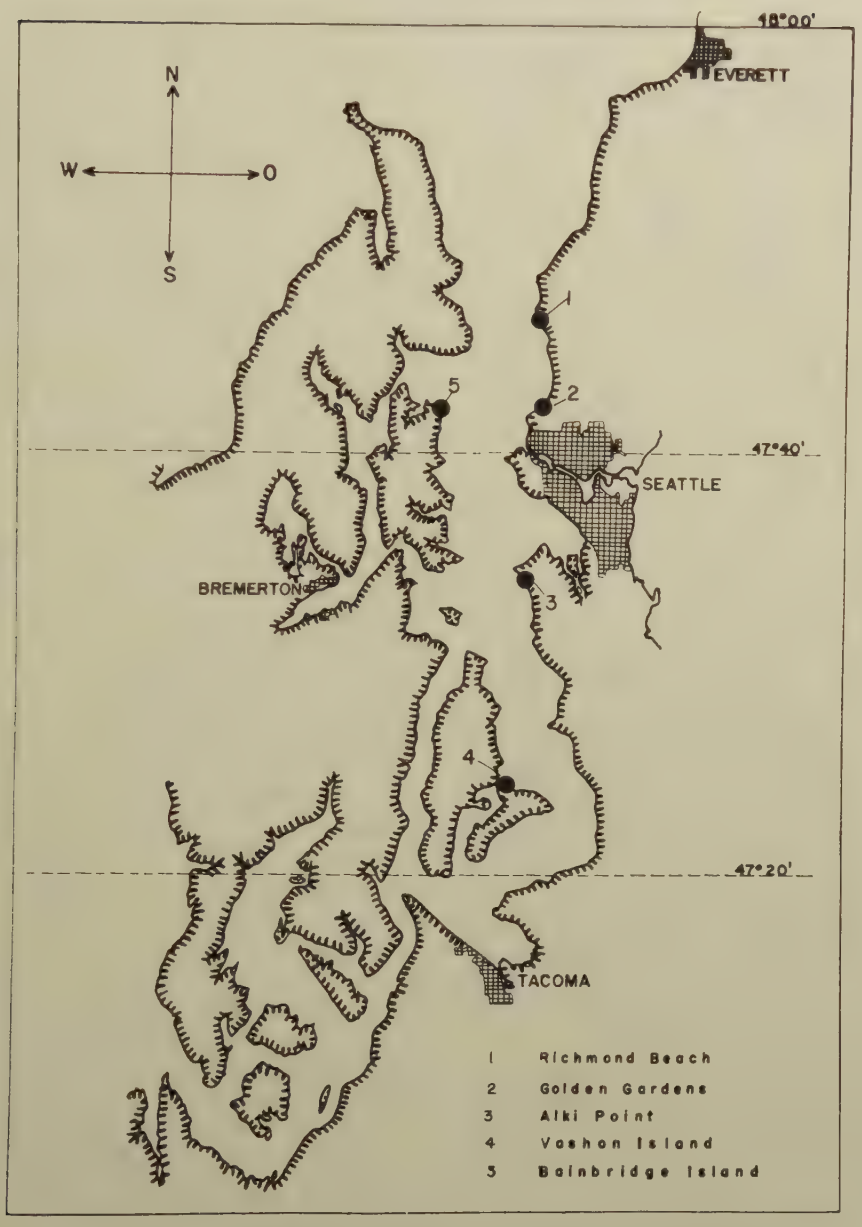
Localities investigated

Five localities were investigated (see Map 1), viz.: 1) *Richmond Beach*, near the railway station: most exposed locality; flat, uninterrupted beach, without boulders; 2) *Golden Gardens*, on the northernmost edge of the public beach: medium fine to coarse sand; between 2 and 4 feet a belt of boulders and rocks, overgrown with *Mytilus* and barnacles; in the lower portion of the intertidal there are patches of *Zostera* in which no samples were taken; above approximately the 3-foot level the beach is steeper than below it; 3) *Alki Point*, between Oregon and Genese Street in West Seattle: medium fine to coarse sand; from 3.5 feet upwards the beach is covered with boulders and rocks, overgrown with seaweeds, barnacles, *Mytilus* etc; in this zone the samples are derived from patches of sand between the boulders; in the lower portion of the beach there occurs the polychaet, *Telepsavus costarum*, apparently an indicator of silty and fine sand; dense algal growth (*Nereocystis*, *Laminaria*, etc.) below low water; 4) *Vashon Island*, in front of the small bridge connecting the two parts of the island: fine to medium fine sand, with boulders in the upper portion of the beach; *Zostera* and *Ulva* in patches in the lower portion; *Telepsavus costarum* reaching up to 5 feet; 5) *Bainbridge Island*, NE point of the island on the beach belonging to the Bainbridge Island State Park: very fine to medium fine sand, very coarse sand around 7-foot level, gravel between 4.5 and 7 feet; *Telepsavus costarum* reaching up to 3–4 feet, patches of *Zostera* reaching up to zero-level.

The position of these five localities with respect to the general configuration of the shore-line and the direction and force of the currents is such that they represent a series of decreasing exposure, Richmond Beach being the most exposed, Bainbridge Island the most sheltered locality. This fact was verified not only by observation in nature (based on the texture of the substrates) but also by the distribution of dyes in the scale-model of Puget Sound which has been built by the Department of Oceanography in Seattle (for this lesson in experimental Oceanography I am indebted to Dr. Clifford A. Barnes).

Sampling

In each locality samples were taken along a transect, roughly between −2 and +8 feet. Five to nine samples were taken per locality, in most cases each sample representing a different level of intertidal height. Each sample was taken during ebbing tide when the relevant level of the beach was still damp, sometimes between two waves but most of the time just above the limit touched by waves. No sampling was undertaken in rough weather. The intertidal height of each sample was determined on the basis of the tide chart of Seattle and vicinity. Sampling was carried out in the following way. The substrate was scooped up with a small beaker, and several sub-samples from the same level, a short distance apart, were emptied into a 1000 cc glass cylinder, until the substrate occupied about one-fourth of the cylinder. Filtered sea water was then added and the cylinder shaken vigorously. After allowing the sand to settle for a few seconds the water was decanted and filtered through very fine bolting silk. The material retained by the bolting silk was put into bottles and the bottles stored in a refrigerator until the contents could be examined. Animals were picked out under a dissecting microscope until it was felt a representative number had been collected.



MAP 1. Puget Sound with the five localities investigated.

For each sample the composition of grain sizes was determined by sieving the (dried) sand through a standard set of Tyler screens, using the following mesh widths: 4760μ (no. 4), 2362μ (no. 8), 1190μ (no. 16), 589μ (no. 30), 295μ (no. 50), 149μ (no. 100). Gravel coarser than 4760μ was discarded. Sand and silt finer than 149μ was collected in a pan and considered as one fraction. The fractions were weighed and the results expressed in per cent of the total weight. In order to obtain a characteristic figure for each sample the "median diameter" was computed on the basis of the cumulative distribution curve of the six fractions (see Krumbein and Pettijohn 1938). Since the fraction below 149μ was not differentiated, the "median diameter" of samples in which

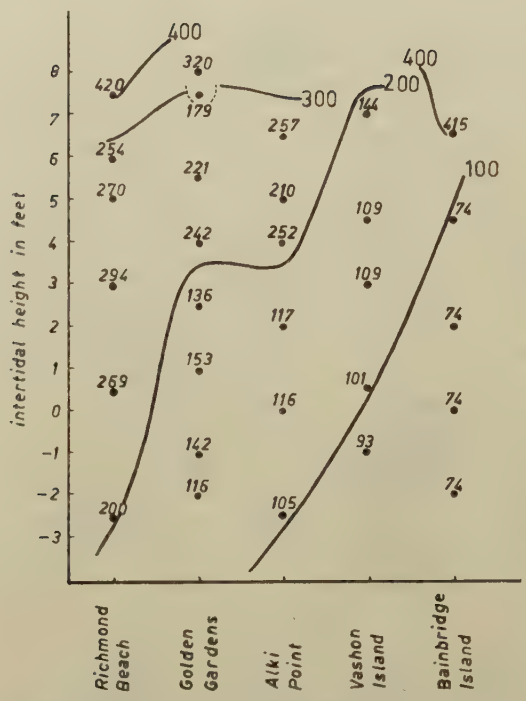


FIG. 1. Position of each sample in the five localities with respect to intertidal height. The five localities are arranged in a series according to their degree of exposure. For each sample the "median diameter" is shown, and the points of approximately equal grain size are connected by curves ("isomegeths").

this fraction made up more than 50% could not be computed. For these samples (of which there were only four) the "median diameter" was arbitrarily fixed at 74μ. Since in this extreme substrate it sufficed to establish the order of magnitude of the mean grain size, its vague determination does not influence any ecological conclusion based on the distribution of the grades of sand.

The substrate

The sorting of sand depends on the strength of water movements. Since the five localities investigated represent a series of decreasing exposure to currents, the mechanical composition of the five beaches can also be arranged in a series, Richmond Beach having on the average the coarsest sand, Bainbridge Island the finest. The sand on Vashon Island and Bainbridge Island consists largely of very fine fractions, but the organic content is low.

Perpendicular to this horizontal gradient, as far as the composition of the substrate is concerned, there runs a vertical gradient due to the tides. Water movements within the tidal zone on most marine shores cause fine sand to be deposited lower downshore than coarse sand since the momentum of the upswell is greater than that of the backwash. There are, however, exceptions to this rule (see also Pennak 1950 for conditions on lake shores).

The mechanical composition of each sample from my five localities is thus determined by two gradients—the horizontal one, represented by the series Richmond Beach-Golden Gardens - Alki Point - Vashon Island - Bainbridge Island, with Richmond Beach being the "coarsest locality," and the vertical one, represented by the series of intertidal levels from high water to low water, with high water being the "coarsest level."

This two-dimensional scheme can be represented by a diagram in which the "median diameter" is taken as an indicator of the composition of the substrate in each sample (Fig. 1). It can be seen that the decrease in mean grain size from high to low water is fairly regular. There is only one major irregularity in Golden Gardens, caused by an accumulation of fine deposits in the upper portion of the beach. The approximate points of equal "median diameter" can be connected, and these "isomegeths"[1] show fairly well the regular distribution of grades of sand in the area investigated. Roughly, coarse material can be found in the upper left, fine material in the lower right of Figure 1. Of great ecological importance is the fact that substrates of similar mechanical composition may be deposited on quite different tidal levels according to the strength of the water movements in the area.

In Tables 1 and 2 the specifications of all the samples are given. The complete list of all the species found in the five localities is given in Wieser (1959).

[1] From ισομεγεθης = of equal size.

TABLE 1. *Intertidal height, median diameter, and date of collection of samples*

Sample no.	Intertidal height (ft)	Median diameter (μ)	Date	Sample no.	Intertidal height (ft)	Median diameter (μ)	Date
Richmond Beach				Alki Point			
1	−2.5	200	5.13.57	16	−2.5	105	5.12.57
2	0.5	269	4.23.57	17	0	116	4.11.57
3	3.0	294	3.17.57	18	2.0	117	2.10.57
4	5.0	270	6.17.57	19	4.0	252	6.13.57
5	6.0	254	4. 7.57	20	5.0	210	12.20.56
6	7.5	384	5. 5.57	21	7.0	207	6.29.57
Golden Gardens				Vashon Island			
7	−2.5	116	10.31.56	22	−1.0	93	2.22.57
8	−1.5	147	6.22.57	23	0.5	101	5.25.57
9	−1.0	142	10.31.56	24	3.0	109	5.25.57
10	1.0	153	1. 9.57	25	4.5	109	2.22.57
11	2.5	136	3.14.57	26	7.0	144	6.29.57
				Bainbridge Island			
12	4.0	242	1.20.57	27	−2.0	74	12.28.56
13	5.5	221	3.14.57	28	0	74	4.26.57
14	7.0	320	10.27.56	29	2.0	74	4.26.57
15	7.5	179	5.17.57	30	4.5	74	12.28.56
				31	7.0	415	6. 2.57

THE INTERTIDAL DISTRIBUTION OF ABUNDANT SPECIES IN RELATION TO THE SUBSTRATE

The distribution of the animals on the beaches is governed partly by the factor of tidal exposure and submersion, partly by the composition of the substrate. In my area of investigation the former factor is constant for all localities, the latter is variable.

In order to understand better the factors which determine the range of distribution of intertidal animals it seems necessary to study the variation of distribution limits under the influence of local factors, the predominant of which is the substrate.

As far as my area of investigation is concerned the hypothesis suggests itself that the upper distribution limit of a species depending on fine sand will be higher up the shore in fine than in coarse sand and, conversely, that the lower distribution limit of a species depending on coarse sand will be lower down the shore in coarse than in fine sand. The following diagrams are designed to test this hypothesis by keeping apart the five localities investigated and by plotting the intertidal distribution of a species in relation to the median grain diameter of the samples in which it was found. All these diagrams are based on Figure 1. Only the most characteristic species are represented in this manner.

Since a general account of the intertidal distribution of *all* species would take up too much space, it will not be given here but can be found as an appendix to my paper on the nematodes of Puget Sound (Wieser 1959).

Hydrozoa

Protohydra leuckarti occurred at practically all tidal levels, but it showed preference for substrates with a median diameter finer than 200μ, so that it occurred higher upshore at Vashon Island than at Golden Gardens (Fig. 2). It might be significant that the species was absent in samples with the finest substrate.

Nematoda

Most of the species can be referred to certain groups as far as their extreme limits of distribution are concerned (Wieser 1959). A great number of species occur throughout the intertidal, amongst them some of the most abundant species of Puget Sound,

TABLE 2. *Relative frequency (percentage) of sand fractions in samples*

Size range microns	Sample								
	1	2	3	4	5	6	7	8	9
4760–2362	3.0	10.6	8.9	8.8	15.2	10.8	–	–	–
2362–1190	6.4	14.3	16.0	11.3	12.3	22.4	+	–	+
1190– 589	12.2	20.2	23.8	22.6	13.9	26.8	0.1	7.2	0.8
589–295	49.0	44.5	43.1	46.0	41.5	33.8	21.5	42.2	44.9
295–149	28.0	10.1	8.1	11.2	16.4	6.2	76.1	44.4	50.4
<149	1.3	0.2	0.1	+	0.7	+	2.2	6.2	3.9
	10	11	12	13	14	15	16	17	18
4760–2362	0.1	0.1	8.2	2.0	16.3	1.1	–	0.7	–
2362–1190	0.3	0.4	9.2	4.3	13.7	1.8	–	0.2	0.1
1190– 589	5.3	5.0	18.8	16.6	22.2	6.7	0.1	0.5	0.5
589– 295	45.2	38.0	45.3	61.8	37.5	59.9	2.9	20.7	23.1
295– 149	45.7	49.6	17.6	15.1	10.1	30.4	90.2	76.0	73.8
<149	3.4	6.9	0.9	0.3	0.2	0.1	6.8	1.9	2.5
	19	20	21	22	23	24	25	26	27
4760–2362	7.8	3.3	5.4	–	0.1	0.1	0.5	0.3	0.7
2362–1190	9.5	4.0	5.6	0.2	0.2	0.3	1.5	0.8	0.8
1190– 589	18.7	10.2	7.5	1.4	1.0	2.7	7.3	1.2	1.3
589– 295	56.4	62.3	58.5	9.4	12.2	19.1	22.8	45.0	3.5
295– 149	7.3	19.0	22.8	58.0	64.9	61.7	40.2	41.9	16.3
<149	0.3	1.2	0.2	31.0	21.6	16.1	27.7	10.8	77.4
	28	29	30	31					
4760–2362	–	0.1	–	11.5					
2362–1190	0.1	0.2	0.1	26.1					
1190– 589	0.2	0.6	0.2	23.9					
589– 295	1.1	4.6	1.8	12.9					
295– 149	6.0	8.9	41.6	24.3					
<149	92.6	85.6	56.2	1.3					

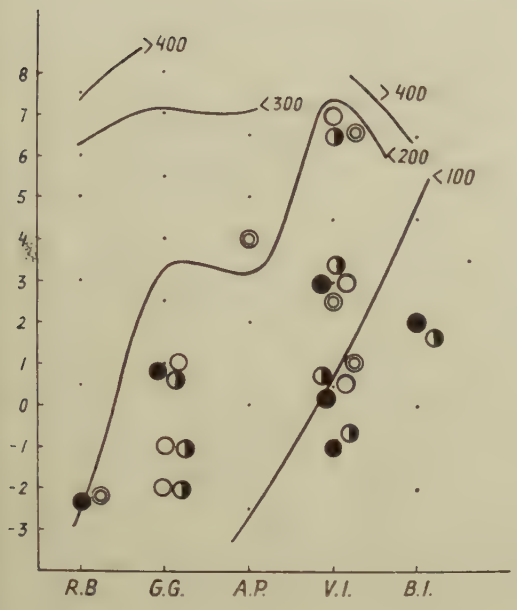
such as *Neochromadora poecilosoma* and *Hyalacanthion multipapillatum*.

The intertidal distribution of species which in some way are dependent on the pattern of distribution of certain grades of sand is illustrated in Figures 3–6. Figures 3, 4, and 5 show a number of species which reach further upshore in the localities with fine substrate than in those with coarse substrate. In some of these species the upper limit of distribution coincides roughly with the 200μ-line, *e.g.* in *Sabatiera* spp., *Theristus wimmeri*, *Mesacanthoides sinuosus*, *Odontophora peritricha*. In other species the upper limit of distribution lies more around the 100μ-line, as in *Enoplus velatus*, *Longicyatholaimus quadriseta*, *Theristus anticipans*.

Figure 6 represents another group of species which are confined to relatively coarse sand and consequently reach further downshore in localities with coarse substrate than in those with fine substrate. This holds particularly for *Enoploides harpax*, *Nudora armillata*, and *Theristus trecuspidatus*. In these species the lower limit of distribution coincides roughly with the 200μ-line.

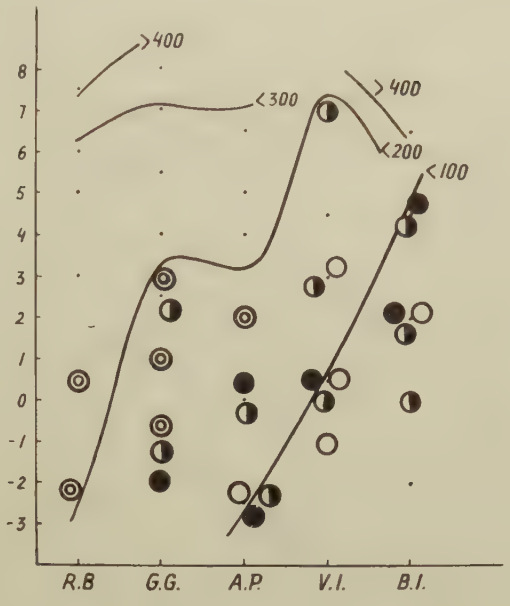
Gastrotricha and Archiannelida

The intertidal distribution of all species belonging to these two groups can be inferred from Figure 7. No species of gastrotrich occurred below the 100μ-line and no archiannelid below the 200μ-line. This contradicts the statement by Pennak (1950, p. 460), that "the horizontal distribution of Protozoa, Turbellaria, Nematoda, Gastro-



○ - Protohydra leuckarti
◑ - Leptochelia dubia
● - Rhynchospio cf. arenincola
◎ - Boccardia sp.

FIG. 2. Distribution of one hydrozoan, one cheliferan and two polychaetes with relation to the "isomegeths." Ordinate: intertidal height in feet.



○ - Enoplus velatus
● - Longicyatholaimus quadriseta
◎ - Theristus wimmeri
◑ - Sabatiera spp.

FIG. 3. Distribution of a set of nematodes with relation to the "isomegeths." Ordinate: intertidal height in feet.

tricha, and Oligochaeta shows no particular pattern" and that "most of these organisms are scattered at random throughout beaches . . ."

Polychaeta

Amongst polychaetes the most abundant species was *Rhynchospio* cf. *arenincola*, the distribution of which resembles that of *Protohydra leuckarti* (Fig. 2). No individuals were found above the 200μ-line. The same holds for the less abundant but equally common *Boccardia* sp. (Fig. 2) and, less characteristically, for *Eteone* sp. The distribution of the other species was more or less patchy (Wieser 1959).

Copepoda

Huntemannia jadensis (the most abundant species on the beaches of Puget Sound) and *Leptastacus* n.sp. occurred throughout the intertidal. A few other species were found in more than one zone, but the occurrence of most of the species was rather sporadic (Wieser 1959).

Two species, *Dactylopodia glacialis* and *Heterolaophonte strömi*, showed a fairly well established preference for substrates with a median diameter finer than 200μ (Fig. 8); they occurred therefore higher upshore at Vashon Island and Bainbridge Island than at Richmond Beach. A third species, *Heterolaophonte discophora*, perhaps displayed a similar mode of distribution, but was found too infrequently.

Ostracoda

Ostracods occurred predominantly below the 3-foot level, that is, in comparatively fine substrate. Of 12 samples in which ostracods were present 10 possessed a median diameter finer than 150μ. Only *Cytheridea papillosa, Leptocythere tenera* and *Cytherura gibba* occurred in sand with a

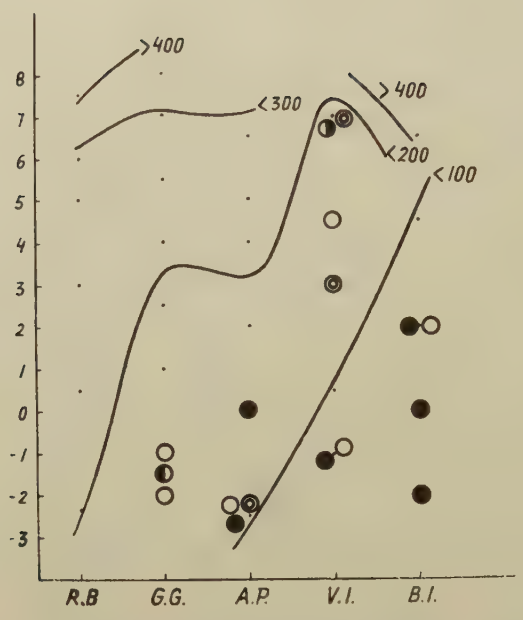


FIG. 4. Distribution of a set of nematodes with relation to the "isomegeths." Ordinate: intertidal height in feet.

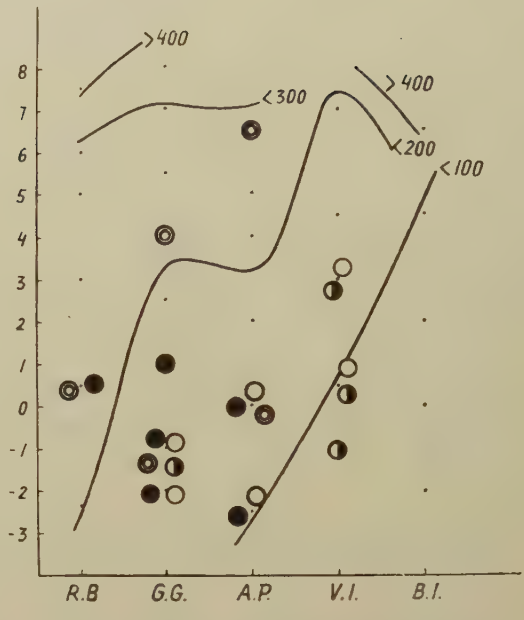


FIG. 5. Distribution of a set of nematodes with relation to the "isomegeths." Ordinate: intertidal height in feet.

median diameter coarser than 180μ. No ostracods occurred above 260μ.

Cumacea

The intertidal distribution of the species of cumaceans can be inferred from Figure 9. It is quite obvious that all three species exhibit a preference for fine substrate. No species surpassed the 200μ-line, except the males of *Cumella vulgaris* which are very active swimmers, while the females and juveniles are not. As far as their intertidal distribution is concerned all three species roughly follow the same pattern.

Other Peracarida

The upper distribution limit of *Leptochelia dubia* roughly coincides with the 200μ-line (Fig. 2).

Exosphaeroma oregonensis occurred in substrates with a median diameter from about 100 to 220μ, and at an intertidal height of 2 to 8 ft.

Amongst the amphipods the most abundant species, *Synchelidium* n.sp., was confined to the lower intertidal (−2.5 to +3 ft). *Corophium salmonis* occurred in the upper intertidal (2 to 8 ft), but only at Vashon Island which is a fine substrate locality.

DISCUSSION

Figures 2 to 9 suggest that the intertidal distribution of at least some of the more common species on the beaches is not so much determined by levels of tidal water as by the pattern of distribution of certain grades of substrate. Species of this sort will, for example, penetrate into the upper intertidal if the preferred substrate occurs there, but they will remain in the lower intertidal if the substrate is confined to this zone.

However, the distribution of such a species cannot always be explained entirely in terms

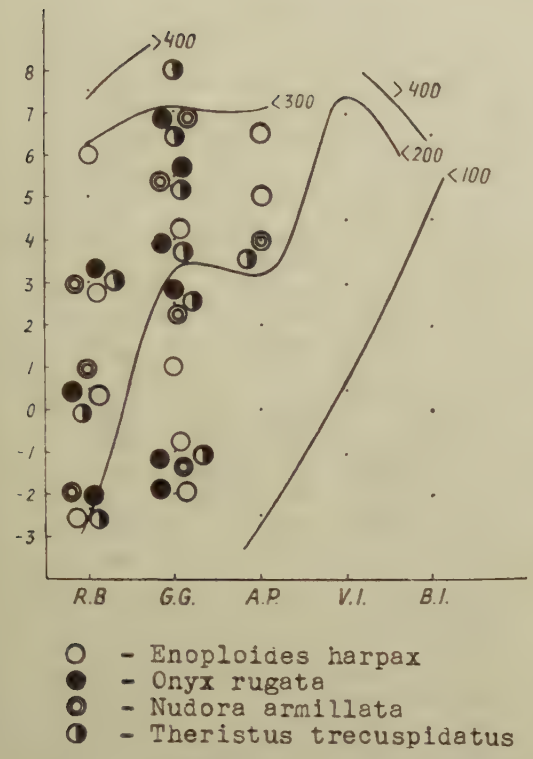


FIG. 6. Distribution of a set of nematodes with relation to the "isomegeths." Ordinate: intertidal height in feet.

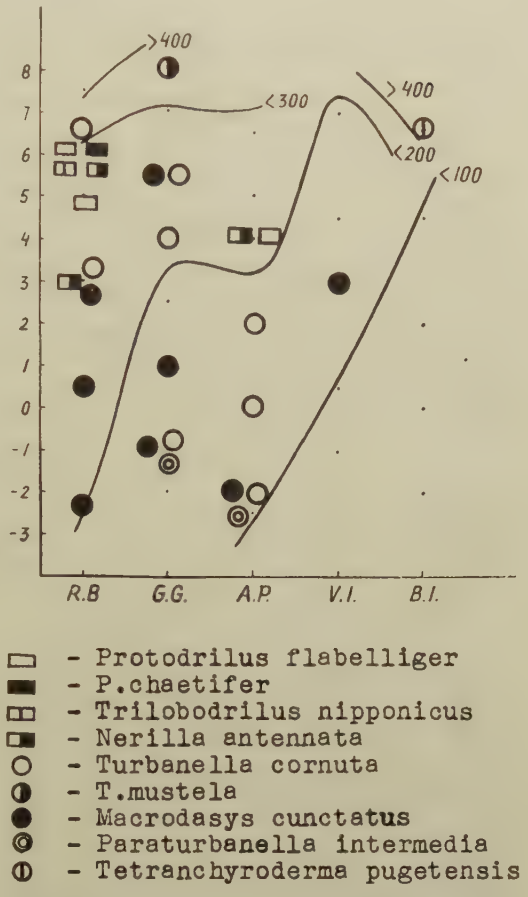


FIG. 7. Distribution of archiannelids (oblongs) and gastrotrichs (circles) with relation to the "isomegeths." Ordinate: intertidal height in feet.

of the presence or absence of certain grades of substrate; very often the availability of the latter is equally important. If, for example, a species does not occur at Richmond Beach this might be due to its preferring grades of sand with a median diameter smaller than 200μ. Consequently the species ought to be restricted *in its whole range of distribution* to samples with a median diameter smaller than 200μ. This certainly is not always the case. The nematode *Anticoma acuminata* is a frequent species in all localities except Richmond Beach, but at Golden Gardens and Alki Point it occurs in samples with a median diameter of 221 and 252μ. The same holds for *Oncholaimium vesicarium* and other species (Wieser 1959).

Such a pattern of distribution might be explained by the fact that at Golden Gardens and Alki Point the lower intertidal consists of relatively fine, the upper intertidal of relatively coarse material (cf. Fig. 1). It will therefore happen quite frequently that waves and rising tide carry animals from the lower into the upper reaches of the beach. In this way species which generally prefer fine sand might occasionally be found—or even become established—in coarse sand. At Richmond Beach on the other hand, the whole intertidal consists of coarse sand. Species preferring fine sand will therefore find less opportunity of being carried in from elsewhere.

A similar explanation might hold for the opposite case. Species which generally prefer coarse sand might be carried into fine sand wherever the intertidal comprises levels of both coarse and fine material, as at Golden Gardens and Alki Point. These

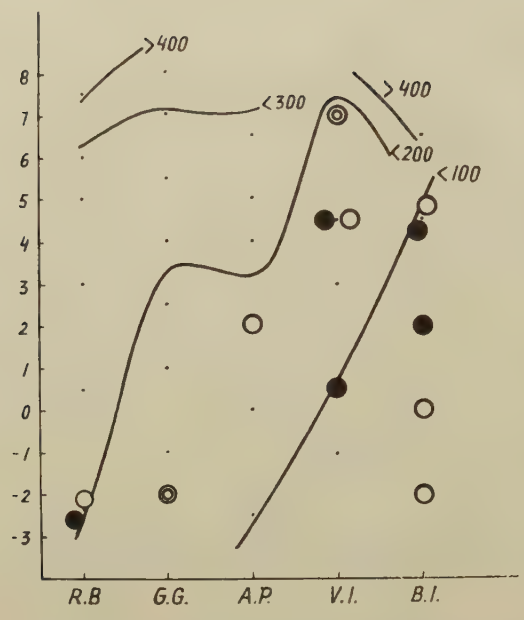


FIG. 8. Distribution of three copepods with relation to the "isomegeths." Ordinate: intertidal height in feet.

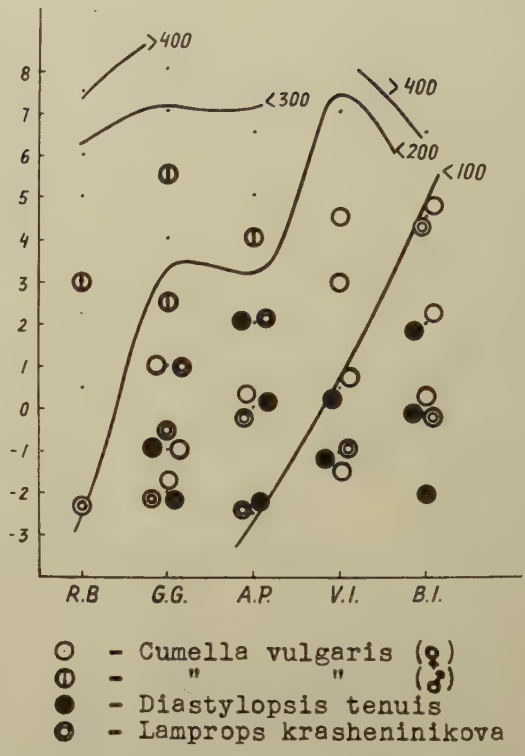


FIG. 9. Distribution of the cumaceans with relation to the "isomegeths." Ordinate: intertidal height in feet.

species will, however, be absent in localities like Vashon Island, in which the intertidal consists entirely of fine material. This assumption would explain the penetration of a number of species, such as *Enoploides harpax*, *Nudora armillata*, *Theristus trecuspidatus*, otherwise confined to coarse material, into the lower reaches of Golden Gardens (Fig. 6).

To me it seems unquestionable that the texture of the substrate exerts a profound influence on the distribution of the fauna, and I cannot agree with the generalized statement by Pennak (1950) that, amongst other factors, the size of sand grains has "no constant relationship to either number or distribution of organisms." The existence of such a relationship is suggested by the work of Davis (1925), Prenant (1932), and Jones (1950), although these authors did not distinguish between the actual effects of grain size and those of the physiographic environment in general of which the grain size is merely an indicator. However, more detailed ecological research within the last ten years indicates that the effect of grain size itself on "either number or distribution of organisms" is quite real and can be of the following types:

1) Sands of different grain size and shape are of different hardness. Bruce (1928) showed that ungraded sand (from Port Erin, Isle of Manx) can be so firmly packed as to have a porosity of only 20% while graded fractions of the same sand had porosities up to 44.7%. These different degrees of packing might influence the distribution of certain animals, or determine the depth to which certain other animals are able to burrow. A possible relationship of this kind is suggested by the work of Ekman (1947) and by that of Chapman (1949) on the thixotropy and dilatancy of marine soil, but the exact nature of the response of animals to different degrees of

hardness of the substrate is not yet established.

2) The horizontal and vertical transport of sand particles depends partly on their size. Some types of sand will be more stable than others, and this might lead to the establishment of characteristic faunas. Sanders (1958) has pointed out that particles with a diameter of 0.18 mm are most easily moved since for them roughness velocity, settling velocity, and threshold velocity are the same, and that sands of that grain size support a fauna characterized by large populations of infaunal filter feeders.

3) Invertebrates that obtain their food by scraping it off sand grains are dependent to a certain degree on the size of the grains. This holds most characteristically for crustaceans some of which alter their mode of feeding if the particles become too small to be handled by means of maxillipeds and maxillae, while too large particles are abandoned altogether. *Cumella vulgaris*, an epistrate feeder on grains between 500 and 150μ, avoids sand grains larger than 500μ, and switches to deposits feeding in still finer substrates (Wieser 1956). Another cumacean, *Picrocuma poecilota*, seems to feed from sand grains with a diameter of 150 to 350μ (Hale 1943). The larger of two amphipods, *Talitrus saltator*, occurs in coarse sand while the small *Talorchestia deshayesii* is present in fine sand (Dahl 1946). This distribution pattern might also be due to the different relationship between mouthparts and sand grains in these two species.

4) The most important characteristic of sands of different grain size is represented by their systems of interstitial spaces. The relationship between the latter and the sand fauna constitutes the main theme of the present paper. The importance of the interstitial spaces for the fauna is based on two facts: firstly, they provide the actual habitat of a specialized component of the sand fauna, and secondly, some burrowing or semisessile animals depend on the circulation of water maintained through the system of interstititals spaces. This has been shown for *Venus mercenaria* by Pratt and Campbell (1956) and for *Branchiostoma nigeriense* by Webb and Hill (1958).

"Critical grain size"

While studying the distribution of larger animals on rocky shores it was found that certain tidal levels proved to be "critical," that is, at these levels greater changes of fauna took place than at others (Coleman 1933, Evans 1947; for application of the "critical level" concept to the microfauna of seaweeds see Wieser 1952). By analogy the question suggested itself if on beaches there might not be "critical gain sizes" which constitute borders or barriers separating major faunal components.

It is, of course, an abstraction to speak of one grain size as constituting a distribution barrier, since the substrate on a beach consists of a mixture of grades of sand. However, as mentioned in the introduction, in almost all samples investigated the sorting was good, and therefore the "median diameter" can be considered a fair representation of the dominant fractions determining the texture of the whole sample. Consequently, the "median diameter" of a given sample can be used as an indicator of the true mechanical composition of this sample.

Keeping this in mind a survey of Figures 2–9 shows that the 200μ-line is a fairly important distribution barrier. This judgement is based entirely on the observer's first impression of the pattern of distribution of the species. To give it weight a statistical study of far more species ought to be made, but the material is insufficient for this purpose.

The 200μ-line approximately constitutes the lower limit of distribution of *Protodrilus flabelliger* and *Nerilla antennata*, of (with the reservations made on p. 190) *Enoploides harpax*, *Onyx rugata*, *Nudora armillata*, and *Theristus trecuspidatus*; it approximately constitutes the upper limit of distribution of *Sabatiera spp.*, *Theristus wimmeri*, *Pareurystomina pugetensis*, *Eurystomina repanda* *Odontophora peritricha*, *Trileptium jacobinum*, *Dactylopodia glacialis*, *Heterolaophonte strömi*, *H. discophora*, *Protohydra leuckarti*, *Rhynchospio cf. arenincola*, *Boccardia* sp.,

Leptochelia dubia, and of all three cumaceans (except the males of *Cumella vulgaris*).

One of the main reasons why sand with a median diameter of 200μ might be an effective distribution barrier is that around this grain size the interstitial spaces which exist in coarser sand are beginning to fill up with fine material. Thus the 200μ-line might approximately separate species that live in interstitial spaces from those that burrow through the substrate, although I doubt whether this holds for nematodes. Most of them are so slender that I suspect them of being capable of moving even between fine sand grains, which faculty they would have in common with the ciliates studied by Faurè-Fremiet (1950). This author found ciliates in sands with a medium diameter of 120μ, and there is no doubt that the protozoans were true representatives of an interstitial fauna, being characterized by great elongation of the body. In fact, some of them (particularly *Geleia orbis* and *Centrophorella fistulosa*) on the basis of Faurè-Fremiet's outline drawings could easily be mistaken for nematodes. Sands with a median diameter of 120μ might be the finest in which interstitial life is possible for any kind of organism.

The figure of 200μ as a grain size of ecological importance was mentioned also by Krogerus (1932) in a study on the relationship between dune sands of various grain sizes and beetles. Krogerus found that in a curve representing the water-holding capacity of sands of various grain sizes a break would occur around the size of 200μ. That is, if exposed to air, sand coarser than 200μ will give off water at a much quicker rate than sand finer than 200μ, the break being due to the discontinuous transition from more or less closely packed find sand in which the capillary forces are high, to coarse sand in which a system of interstitial spaces is developed and the capillary forces are comparatively low. This is in excellent agreement with the finding of Webb (1958) that in sand consisting of grains larger than 0.2 mm drainage of water is rapid, "not exceeding 11 minutes for a 10 cm column . . . while the drainage time through grains of 0.2 to 0.1 mm in diameter was more than 25 hours." In consequence, the lancelot, *Branchiostoma nigeriense*, which is a ciliar feeder and dependent on strong water circulation, if given choice of various substrates always chose those of high permeability, that is, substrates with grains between 0.2 and 0.3 mm. An addition of 25% of fine sand apparently obstructed the water circulation through the interstitial spaces and rendered the substrate unfavorable to the animals. The mystacocarid *Derocheilocharis remanèi* also occurs in sands with a median diameter between 0.2 and 0.3 mm (Delamare Deboutteville 1954).

Distribution of locomotor types

Animals living in sand can move along either by sliding within the interstitial spaces or by pushing and burrowing through the medium. The former mode of locomotion is possible only in sand coarse enough to permit the development of an interstitial system (see Remane 1933, 1940). The latter mode ought to be most common in fine sand. The distinction between the two types is by no means sharp. Animals which in fine sand are burrowers might in coarse sand slide through interstitial spaces. Several copepods, for example, are likely to represent such intermediary types.

It would be interesting to know the distribution of these locomotor types with respect to the texture of the substrate, particularly with respect to the grain size of 200μ just mentioned. An attempt to summarize the pertinent data has been made in Figure 10. The unit in the diagram is the number of species in each sample for all groups investigated except nematodes. Most of the nematodes have to be considered as inhabitants of interstitial spaces, but as already mentioned they are so slender as to represent a group apart from all others. Moreover, Gerlach (1954) has shown that some species are burrowers, and although it is quite easy to recognize characteristic species of this type by their blunt shape it is by no means impossible that a number of species of less extreme shape may also employ this mode of locomotion. The decision as to which species uses which mode of

locomotion is particularly difficult to reach in this group.

In Figure 10 the following species were considered as interstitial "sliders": all archiannelids, all gastrotrichs, and amongst copepods *Arenosetella* spp., *Pararenosetella gracilis*, *Leptastacus* n.sp., *Paraleptastacus* n.sp., and *Paraleptocaris* n.sp. (based on verbal information by W. Noodt). All other species apart from the nematodes are burrowers.

It can be seen that above the 200μ-line sliders are represented in all samples and that the number of burrowing species rarely exceeds six per sample. Below the 200μ-line there also occur sliders in a number of samples but most of them at Golden Gardens and Alki Point, where both coarse and fine sands are in close proximity (see p. 189). In most of the samples below the 200μ-line there are more than 10 burrowing species. No sliders were found below the 100μ-line.

These results enable us to see more clearly another way in which grain size might affect the distribution of animals on the beach. As was shown by Webb (1958), when the tide is receding the level of interstitial water will fall more rapidly in coarse than in fine sand. Consequently, in coarse sand there will be a premium on those animals that are able to follow the level of water as rapidly as it falls. This will be easiest for animals adapted to life in interstitial spaces since sliding in these spaces is virtually unimpeded and, theoretically at least, the animals should be able to stay close to the falling water level down to any depth (copepods, according to Pennak 1942, can still be found at a depth of 25 cm). Burrowing animals would find this much more difficult, since even a few centimeters below the surface the resistance of the medium is considerable.

In fine sand, on the other hand, capillary forces are greater, and the water level will fall more slowly when the tide is receding. Burrowing and slowly moving animals will therefore be able to stay close to the falling water either by burrowing downwards into the sand or by following the ebbing tide towards the low water mark.

Thus, put in another way, we may distinguish on the beach between "vertical migrants," animals that move vertically within the sand layers, and "horizontal migrants," animals that move horizontally on the beach between high and low water mark. In the latter case the mode of locomotion may be either by crawling or by swimming. The driving force for each animal is to stay within a sand layer of optimum humidity. A similar distinction between "vertical" and "horizontal" migrants was established by Watkin (1941) and Remane (1940, p. 107). To the former type belong all interstitial forms, to the latter agile and large animals like amphipods and cumaceans, but perhaps also smaller forms like ostracods.

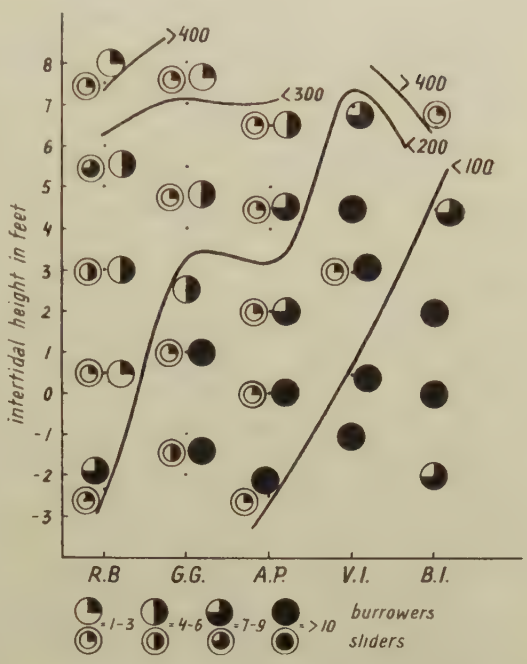


FIG. 10. Distribution of locomotor types with relation to the "isomegeths." Simple circles = burrowers; double circles = interstitial sliders. The number of species belonging to each type is indicated by the varying size of the black segments in the circles.

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Seasonal Changes in Bluegill Metabolism[1]

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ABSTRACT

A one-year study of bluegill metabolism in a California reservoir indicated that field determinations of oxygen uptake rates and multivariate analyses of results with respect to weight, activity, and temperature during each season were feasible in the form,

$$Y_e = \text{constant} + b_1X_1 + b_2X_2 + b_3X_3,$$

where Y_e is the expected log milligrams of oxygen consumed per hour, X_1 is the log weight in grams, X_2 is the swimming velocity in meters per minute, X_3 is the temperature in degrees centigrade, and where b_1, b_2, and b_3 are the respective partial regression coefficients. By delimiting "seasons" on the basis of thermal and related limnological characteristics, the data yield the equations:

Apr.–June	$Y_e = -0.6954 + 0.8036X_1 + 0.0250X_2 + 0.0069X_3$
July–Aug.	$Y_e = -2.1951 + 1.0621X_1 + 0.0138X_2 + 0.0567X_3$
Sept.–Dec.	$Y_e = -0.9242 + 0.8494X_1 + 0.0142X_2 + 0.0198X_3$
Jan.–Feb.	$Y_e = -1.9310 + 0.8624X_1 + 0.0427X_2 + 0.1153X_3$

The multiple correlation coefficients for these equations are highly significant as are all the partial regression coefficients, excepting the low spring $b_3 = 0.0069$.

The weight relationships expressed by b_1 are remarkably close to Krogh's constant of approximately 0.85 except in the summer when the calculated higher rate may be due to the spawning condition of the larger fish and the post-spawning condition of the smaller fish.

There are similar relationships of respiratory rates with respect to swimming movements over the spring, summer, and autumn seasons, but during winter it appears that relatively higher oxygen consumption is required for comparable swimming activity.

The respiratory rates with respect to temperature appear to be of the same order during spring and autumn when they are lower than in summer and much lower than in winter. The high respiration-temperature regressions in winter, and possibly in summer, may be due to excessive spontaneous activity not reflected as swimming motion at temperature extremes.

Females during each season have higher metabolic rates than the males over the range of temperatures and activities encountered, but the differences are not statistically significant.

Groups of fish during the spawning season have higher than average respiratory rates than do single fish, other conditions being equal. Limited data suggest that social facilitation exists during the summer after spawning.

Variations in Felt Lake limnological conditions are considered from the standpoint of about a 50 per cent reduction in volume from spring to autumn, the absence of a well developed littoral zone, and the rather drastic spring to autumn and winter declines in bottom fauna and average weights of the larger fish. A comparison of the above regressions over average spring and autumn conditions reveals that metabolic rates would be about equal,

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although in spring the fish grow rapidly and spawn, while in autumn and winter they lose weight. Similarly the calculated winter metabolic rates are higher at comparable temperatures than those of spring or autumn due possibly to cold adaptation.

While the potential growing season in Felt Lake would be about twice as long as that for the northern portion of the native bluegill range, the growth rates are of the same order. The biological productivity of the bluegill in Felt Lake and similar western U. S. waters is interpreted to be much poorer than in eastern areas because of the longer growing season without adequate food (especially bottom fauna) production and because there appears to be no adaptive mechanism for lowering the metabolic requirements during seasons of poor food supplies.

INTRODUCTION

The purpose of this study is to describe quantitatively seasonal changes in respiratory rates of the bluegill, *Lepomis macrochirus* (Centrarchidae), in relation to seasonal limnological variations of Felt Lake, a small California reservoir.

Included among the physiological and limnological factors which influence the respiratory (oxygen consumption) rate are: the direct temperature effect, effect of temperature acclimation, oxygen and carbon dioxide content of the water, body weight, body fat content, cruising speed or activity, recency and amount of feeding or degree of starvation, extent of social facilitation, sex, sexual development and spawning, excitement, injury, shock, parasitism, disease, and perhaps many other factors. Fry (1957) has given a comprehensive account of the literature dealing with the effects of these factors on fish respiration and metabolism, and Wohlschlag (1957) has noted that body weight, swimming velocity, and temperature accounted for most of the variability in oxygen consumption rates of an arctic whitefish. Gerking (1954) has emphasized the importance of fish metabolism data by showing how metabolism and biological production are related in a natural environment.

Customarily respiratory rates are expressed as log oxygen consumed per hour, and metabolic rates are expressed as log oxygen consumed per hour per kilogram of body weight, either in total weight or in protein weight units. Respiratory or metabolic rates are also expressed as "standard" rates which approach basal rates, as "active" rates which pertain to fish with sustained "maximum" swimming velocities, and as "routine" or intermediate rates which pertain to fish exhibiting spontaneous activity other than swimming (Fry 1957). Most of the published data on fish metabolism refer to determinations for fish carefully acclimated to controlled laboratory conditions, although Pritchard (1955) has made determinations for two small marine species held in concrete cisterns, and Wohlschlag (1957) has made determinations on whitefish in their natural habitats. There is very little information available to suggest to what extent metabolism of fish varies with seasonal limnological conditions.

On the assumption that bluegills in their natural state were "acclimated" to currently existing limnological conditions, a field study was set up at Felt Lake to evaluate any seasonal changes that might occur in the respiratory rates with respect to body weight, various swimming rates, temperature, sex, and grouping.

DESCRIPTION OF FELT LAKE

Felt Lake is a reservoir, retained by an earth-fill dam, on the Stanford University campus. It is about five miles west of Palo Alto, California, at Lat. 37° 24′ N and Long. 122° 14′ W. The inflow is via a one-half mile diversion ditch from Los Trancos Creek. Above the diversion the drainage basin consists of about 5.4 square miles; immediately tributary to the lake, the drainage basin consists of about 110 acres. Rainfall to the extent of about 15 inches per year occurs mostly from November through March. These winter rains maintain the reservoir at a 300 million gallon capacity with a surface area of 46 acres until about May. The level declines with irrigation needs about 10–12 feet by late summer when the volume is about 125 million gallons. In 1956–57 the lake was full from March to June. From June to October the level dropped regularly a total of 10 feet.

During the very dry November and December period the level dropped only one foot. The lake filled during January and February, 1957.

The 1956–57 surface water temperatures ranged from 8.0–26.5°C. Formation of a partially stable thermocline in summer and partial hypolimnetic oxygen depletion can occur; however, the oxygen content of the surface waters remained near saturation levels during 1956–57.

From spring through autumn the littoral areas tend to have a fairly dense growth of weeds which follow lakeward the declining water level. Consequently the littoral fauna, which reaches its peak in late spring, is poorly developed in comparison to lakes with constant levels. It is estimated that an average volume of stomach contents for spring and early summer would be at least 1–2 cc. Frymire (unpublished M.S. thesis) has shown that the bottom fauna as a food supply for bluegills whose fork lengths were 125 mm and over is severely curtailed by autumn when the average volume of stomach contents declined to 0.20 cc per fish. By early winter the average volume had declined to 0.17 cc, with mollusk and plant remains making up a large portion of the stomach contents. By late March insects were again important foods, and the average volume of stomach contents rose to 0.52 cc.

On the basis of several hundred length and weight measurements taken in early May and again in early November, 1956, for fish in the fork-length classes of 125–149 mm and 150 mm and over, it is apparent that there is a marked decline in condition (weight per unit length). In the spring the smaller class had respective averages of length and weight of 138.4 mm and 54.8 g, while the respective averages for the larger class were 158.5 mm and 90.1 g. The corresponding data for autumn were 139.7 mm and 50.5 g, and 157.4 mm and 75.5 g.

The bluegill is the dominant species in the lake. From unpublished population dynamics studies, the growth and recruitment is rapid only in the spring and early summer when the population of fish whose fork lengths are 125 mm and over varies from 20 to 25 thousand. Following the late summer and autumn periods of poor growth and high mortality rates accompanied by a high incidence of parasitism, this population is reduced by as much as one-half. The adverse winter conditions persist until March warming when the smaller fish, especially, start to grow rapidly and begin to spawn in April to May. The larger bluegills start to grow somewhat later and begin to spawn from May to June and continue spawning as late as August.

Felt Lake also has a small population of largemouth bass (*Micropterus salmoides*) and very small populations of the brown bullhead (*Ictalurus nebulosus*) and the sculpin (*Cottus asper*).

FIELD PROCEDURES

Bluegills were obtained by seining or trapping in surface waters. They were retained in a live car for a period of at least one hour before measurement of their respiratory rates in a respiration chamber. All respiratory measurements were carried out in the field from March, 1956, through February, 1957.

The respiration chamber, described by Wohlschlag (1957), is a circular, completely enclosed, transparent plastic chamber which can be rotated. Rotation was effected by suspending the chamber from an inverted, spring-wound, phonograph motor whose velocity could be controlled by a manually regulated governor. In the earlier determinations the motor and chamber were suspended from a four-legged derrick set up in shallow water near shore. Later determinations were made when the rig was suspended through a deck hatch of a raft located at the center of the lake. Approximate dimensions of the chamber are: outside diameter, 45 cm; inside diameter, 25 cm; depth, 10 cm; and volume, 10,400 ml. A complete revolution by a swimming fish would equal a distance of about one meter.

For each determination fresh surface lake water was added to the chamber through a funnel-like reservoir and connecting plastic tube. Fish were introduced through a lid which was then sealed. Runs were made with single large fish due to space limitations within the chamber, but smaller fish were

often used in groups of two to eighteen depending upon their size and availability. Bubbles were removed through the connecting tube of the reservoir and the entire chamber was lowered by a pulley system so that it was barely immersed in the lake. The chamber was then rotated at whatever approximately constant velocity the fish would tend to maintain.

Samples of water for oxygen analyses were withdrawn through an outlet tube after the chamber was raised above lake level. Simultaneously fresh water from the reservoir replaced the withdrawn water without the introduction of bubbles. These samples were taken initially and at 10- to 15-minute intervals over a period of one-half to one hour. The oxygen contents of the samples were determined by the modified Winkler method. Except for the spring months of 1956 all oxygen analyses were completed in the field.

While respiratory rate determinations were underway, temperature and rotation velocities were periodically recorded; after the run these data were averaged, the fish were weighed, and notes were taken on sex, sexual development, stomach contents, and visible parasites.

RESULTS AND COMPUTATIONS

Field data

To determine the rate of oxygen consumption, or respiration, the oxygen content of the chamber was calculated for the time of each water sampling. A systematic correction was made to allow for the additional oxygen introduced into the chamber by the fresh water which replaced the withdrawn water. For each respiration run the corrected oxygen contents of the chamber were plotted against elapsed time. The least squares calculation of the slope of the best straight line through these four or five points yielded the respiratory rate in milligrams of oxygen consumed per hour for the total weight of the fish. In Appendix Tables A–D are listed the respiratory rates in logarithms (Y), the log total weights (X_1), the swimming velocity in meters per minute (X_2), the temperatures (X_3), and the metabolic rates (Y_1) expressed as log milligrams of oxygen consumed per hour per kilogram. The four "seasons" into which these data are separated are arbitrary delimitations of: (1) a period of rapidly increasing temperature and spawning activity in spring; (2) a warm summer period with declining water levels; (3) a long, late summer and autumn period of low water level characterized by declining temperature and benthos and by a rising incidence of starvation and parasitism; and (4) a cold winter period of winter rains with high and turbid waters.

Multiple regressions

The interrelationships of the expected log oxygen consumption (Y_e), which is dependent on the log body weight in grams (X_1), the activity (X_2) in meters per minute, and temperature (X_3) in degrees centigrade, can be expressed as a multiple regression (Wohlschlag 1957). Routine procedures in most standard statistics books, *e.g.*, Goulden (1952) and Snedecor (1956), are available for the calculation of the multiple regression,

$$Y_e = \text{constant} + b_1 X_1 + b_2 X_2 + b_3 X_3,$$

where b_1 is the partial regression coefficient of Y on X_1 with constant X_2 and X_3, b_2 is the partial regression coefficient of Y on X_2 with constant X_1 and X_3, and b_3 is the partial regression coefficient of Y on X_3 with constant X_1 and X_2.

The regressions based on data in Appendix Tables A–D have been calculated in two ways. The first method involved the values Y and X_1 as tabulated for the total oxygen consumed for the total weight of fish in the chamber; the second method involved Y as the log average milligrams of oxygen consumed per hour per fish and X_1 as the log of the average weight per fish in cases where more than one fish was enclosed in the chamber. Regressions based on the first method are given in Equations 1–5 in Table 1, while the regressions calculated on a "per fish" basis are given in Equations 6–11. Calculated values have been rounded to four decimal places. Reasons for the various seasonal breakdowns are discussed below. Other useful regression statistics

TABLE 1. *Regression equations for Felt Lake bluegills relating log oxygen consumption rates (Y_e) to log body weight (X_1), swimming velocity (X_2), and temperature (X_3)*

Season	Number of determinations	Regression equation
		For total weights
March–June	31	$Y_e = -0.7248 + 0.8232X_1 + 0.0158X_2 + 0.0132X_3$ (1)
Apr.–June	23	$Y_e = -1.6459 + 1.3132X_1 + 0.0167X_2 + 0.0107X_3$ (2)
July–Aug.	22	$Y_e = -1.9808 + 0.9571X_1 + 0.0140X_2 + 0.0557X_3$ (3)
Sept.–Dec.	40	$Y_e = -0.6912 + 0.7167X_1 + 0.0107X_2 + 0.0235X_3$ (4)
Jan.–Feb.	22	$Y_e = -1.7517 + 1.2167X_1 + 0.0091X_2 + 0.0439X_3$ (5)
		For average weights
Apr.–June	23	$Y_e = -0.6954 + 0.8036X_1 + 0.0250X_2 + 0.0069X_3$ (6)
July–Aug.	22	$Y_e = -2.1951 + 1.0621X_1 + 0.0138X_2 + 0.0567X_3$ (7)
Sept.–Dec.	40	$Y_e = -0.9242 + 0.8494X_1 + 0.0142X_2 + 0.0198X_3$ (8)
Jan.–Feb.	22	$Y_e = -1.0603 + 0.9630X_1 - 0.0002X_2 + 0.0251X_3$ (9)
Jan.–Feb. (active fish)	15	$Y_e = -1.9310 + 0.8624X_1 + 0.0427X_2 + 0.1153X_3$ (10)
Jan.–Mar.	30	$Y_e = -0.6799 + 0.8235X_1 + 0.0063X_2 + 0.0107X_3$ (11)

TABLE 2. *Felt Lake bluegill oxygen consumption regression statistics*
(See Text and Table 1.)

Equation	Degrees of freedom	Standard error of estimate	Multiple correlation		Standard errors of estimate, s_b, for partial regression coefficients					
					Log wt., x_1		Activity, x_2		Temp, x_3	
			$R_{Y\cdot123}$	P	s_{b1}	P	s_{b2}	P	s_{b3}	P
1	27	0.1351	0.7010	<0.01	0.1872	<0.001	0.0060	<0.02	0.0099	<0.20
2	19	0.1472	0.6070	<0.05	0.4786	<0.02	0.0082	<0.10	0.0123	<0.40
3	18	0.1021	0.8414	<0.001	0.2413	<0.001	0.0063	<0.05	0.0170	<0.01
4	36	0.1662	0.5867	<0.01	0.3174	<0.05	0.0067	<0.20	0.0071	<0.01
5	18	0.1742	0.5970	<0.05	0.4336	<0.02	0.0170	<0.70	0.0327	<0.20
6	19	0.1737	0.9067	<0.001	0.1274	<0.001	0.0113	<0.05	0.0161	<0.70
7	18	0.0582	0.9914	<0.001	0.0337	<0.001	0.0035	<0.01	0.0095	<0.001
8	36	0.1631	0.8468	<0.001	0.1037	<0.001	0.0070	<0.05	0.0076	<0.02
9	18	0.1932	0.6495	<0.05	0.3432	<0.02	0.0188	>0.90	0.0366	<0.60
10	11	0.1119	0.8787	<0.001	0.2050	<0.01	0.1054	<0.02	0.0316	<0.01
11	26	0.1547	0.5943	<0.01	0.2393	<0.01	0.0084	<0.50	0.0093	<0.30

for these equations are given in Table 2 with probabilities (P) indicated for the overall multiple correlation ($R_{Y\cdot123}$) and the individual partial regression coefficients (b_1, b_2, and b_3). Probabilities are referred to tabulated values at levels 0.001, 0.01, 0.02, 0.05, and 0.1 to 0.9 by tenths.

Sex and metabolic rates

While the experiments on single fish were not designed specifically for a comparison of male and female metabolism, the following schedule for the comparisons of the Y_1 (log O_2 consumption rate per unit body weight) in the Appendix is of interest:

Season	Average Y_1	
	Males	Females
Apr.–June	2.2378	2.2590
July–Aug.	2.2942	2.4693
Sept.–Dec.	2.1674	2.2209
Jan.–Feb.	2.1006	2.1598

In all seasons the females have the higher metabolic rates, but none of the differences between sexes is statistically significant.

Effect of grouping

Indirect comparisons between oxygen consumption rates (Y) of single fish and of

two or more fish may be made by utilizing the Table 1 regressions (based on average weights) to calculate the expected values of oxygen consumption for the experiments in which groups of fish were used. A comparison of expected Y_e and observed Y_o values is then possible.

For example, in the April–June period Y_e values may be calculated from Equation 6 (Table 1) and compared with the observed rates Y_o in Appendix Table A as follows:

Experiment No.	Y_o	Y_e
11	1.143	1.050
18	1.312	1.118
19	1.258	1.056
20	1.332	1.114
23	1.277	1.143
26	1.088	0.970
27	1.034	0.987
28	1.352	1.118
29	1.336	1.205
30	1.241	1.078

All ten observed values are higher than expected.

By utilizing Equation 7 (Table 1) and the data of Appendix Table B, the following comparisons for the July–August period obtain:

Experiment No.	Y_o	Y_e
47	1.075	1.207
48	1.481	1.569
49	1.268	1.277
50	1.300	1.258

Only the comparison for Experiment No. 50 indicates a higher value for the Y_o, and this is very likely explained by the agitation among the three fish which included a small ripe male and a small ripe female.

The September–December comparisons based on Equation 8 and Appendix Table C data are:

Experiment No.	Y_o	Y_e
56	1.353	1.111
57	1.105	0.872
64	1.192	1.170
65	1.268	1.161
69	1.100	1.140
76	0.989	1.053
78	1.108	1.078
95	1.157	1.065
96	1.118	1.196

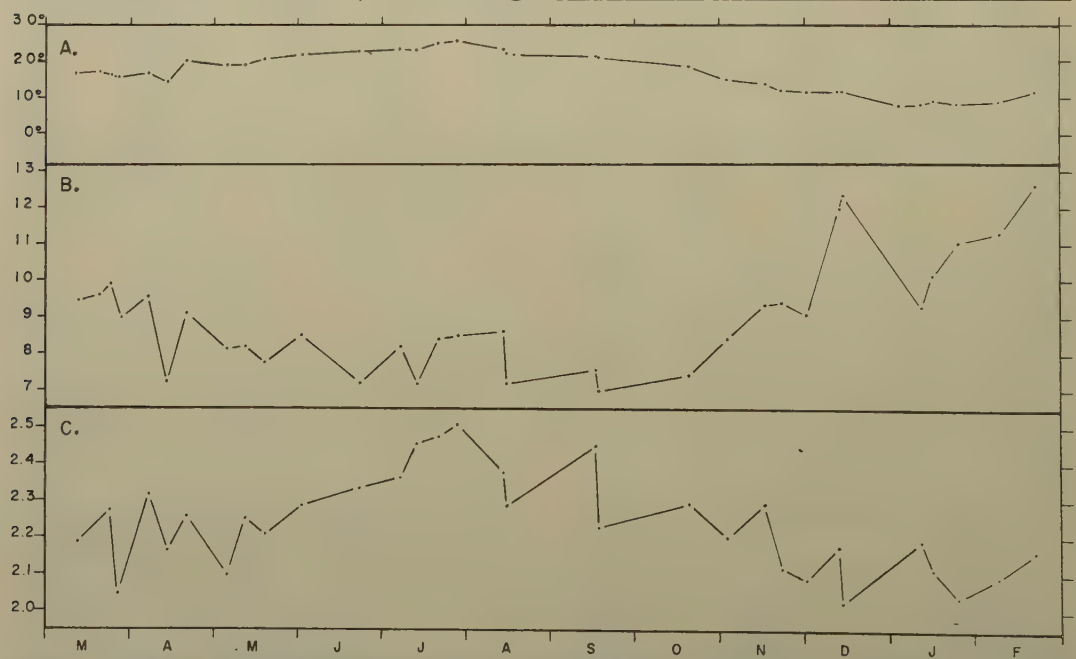

FIG. 1. Trends of 1956–57 Felt Lake averages for selected days. Surface temperatures in degrees centigrade (Panel *A*), milligrams oxygen per liter at surface (Panel *B*), and log milligrams oxygen consumed by bluegills per hour per kilogram (Panel *C*).

That six of the nine Y_o are higher than expected hardly provides a significant indication of difference.

The January–February data contained only one group determination. The March data were inadequate due to temperature variations.

Limnological conditions

Shown in Figure 1 is the trend of averages of Felt Lake surface temperatures and oxygen contents and of the bluegill metabolic rates for the days during 1956–57 when field determinations were made.

EVALUATION OF DATA

Methodology

Fry (1957) gives excellent critiques of laboratory methods for measurement of respiration or metabolism. Wohlschlag (1957) gives a critique of the field methodology pertinent to this study. There appears no evidence to indicate that manipulation of gear or determination of the oxygen consumption rates should involve any systematic biases.

The oxygen content of the chamber declined linearly on the average during each run, and ordinarily did not drop below about 70 per cent of saturation. There was thus no indication that respiratory rates decreased with time due to lower oxygen tension as might be expected with relatively large fish (Job 1955, Fry and Hart 1948a, Fry 1957) or with relatively very active fish (Graham 1949). The initial oxygen consumption rate during the first 10–15 minutes was not higher than average due to excitement (Black *et al.* 1939), nor did the fish show any symptoms of irritability except in winter. In January–February some of the inactive fish tended to have excessively high respiratory rates (see Equations 9 and 10, Table 1). Otherwise the bluegills were fairly placid whether swimming or not.

There is the possibility that hour-to-hour oxygen consumption rates could vary with endogenous daily cycles as demonstrated by Clausen (1936) and others for standard metabolic rates. However, all observations in this study were from daylight hours and are quite likely comparable. Another source of hour-to-hour variation occurred during the March, 1956, determinations when the respiration chamber was in shallow water, and shoreline water temperatures increased rapidly during the day to the extent of 2–3° above the temperatures to which the fish were acclimated. From Fry's (1957) review of thermal stimulation and acclimation data related to metabolic rates, it would appear that the March data are apt to be unusually variable. A comparison of the January–February with the January–March regressions (Equations 9 and 11) or a comparison of the March–June with the April–June regressions (Equations 1 and 2) based on Table 2 statistics indicates the pronounced effects of the March data. Hence it would seem desirable to omit these data from further consideration.

From Figure 1 it will be observed that April and May metabolic rates fluctuated with surface water oxygen contents. The significance of this relationship is unclear. Since the oxygen contents for each run were initially above 7 mg O_2/L, it is unlikely that respiratory dependence on oxygen would be involved.

Linearity

Not only would interseasonal variation be expected for some of the partial regression coefficients, but there would also be expected intraseasonal variation over the individual seasonal ranges of limnological conditions. Should intraseasonal variations be pronounced, curvilinear and time-series analyses of the data would be necessary. However, there are several reasons why the linear analysis used here is satisfactory.

It is well known, at least since the time of Krogh's (1916) studies, that the log oxygen consumption rate plotted against log weight is nearly linear. There may be some exceptions at extremes of size ranges (Zeuthen 1947, 1953), temperatures, activities, and low oxygen levels. Fry (1957) discusses these interrelationships at length. Since extremes were not encountered, and since the partial regressions coefficients b_1 are highly significant (Table 2), there is good reason to consider the log oxygen

consumption rate-log body weight relationships essentially linear.

Linearity of the log oxygen consumption-swimming velocity rate is harder to evaluate. A few workers, *e.g.*, Spoor (1946) and Sullivan (1954), have utilized "spontaneous activity" measurements of relatively stationary fish. Spoor's data would be essentially linear if oxygen consumption rates were plotted logarithmically, while Sullivan's data would not. Most other workers have considered activity as being negligible in determinations of "standard" or "resting" metabolism or as being maximal on a sustained basis. In the Appendix it will be noted that the range of activity is small and that the maximum swimming velocities are much lower than would be expected had the fish been stimulated to maximum possible activity. Because the range of swimming velocities is small and of a low order the log oxygen uptake-velocity regressions appear essentially linear, even though the relationships over a wider range of possible activities and temperatures would be curvilinear (Fry and Hart 1948b). One pronounced exception to a linear rate did occur during the January–February period when some of the non-swimming fish appeared especially irritable. For the 22 observations summarized by Equations 5 and 9 (Table 1) the partial regression coefficients b_2 and the multiple correlation coefficients have a lower order of probability (Table 2) than they do when the seven inactive fish (Appendix Table D) are not considered as in Equation 10. The observed oxygen consumption rates for five of the seven non-swimming fish are higher than the expected values from Equations 9 or 10. Besides appearing irritable and refusing to swim, four of these seven fish were definitely thinner than the average for this season when their physiological condition was already poor. Thus it would seem desirable during the colder periods to consider for regression analysis on a linear basis only the fish whose activities can be measured.

Besides its effect on activity, temperature also influences directly the oxygen uptake rate of poikilotherms. From data based on standard and routine metabolism assembled by Fry (1957) it would appear that the log metabolic or respiratory rates plotted against temperature might be at least slightly curvilinear over a wide temperature range. The annual temperature range at Felt Lake is fairly small, and by breaking up the data into seasons, each with a still smaller temperature range, it is reasonable that the log oxygen consumption-temperature regressions b_3 are essentially linear.

From the above considerations on the effects of log weight, swimming velocity, and temperature variables on the log oxygen uptake, from the relatively high multiple correlation coefficients, and from the individual partial regression coefficients with small standard errors (Table 2) it may be concluded that these variables may justifiably be treated on a linear basis.

Total versus average weights of fish

For the determinations involving more than one fish in the chamber, a question arises on the soundness of treating the data either in terms of the total oxygen consumed for the aggregate weight of the fish or in terms of the average amount of oxygen consumed per average weight of fish. If totals are used the regressions (Equations 1–5) are restricted to smaller weight ranges than is the case when averages are used (Equations 6–11). If a very small single fish were in the chamber, its oxygen consumption rate would have been too small for adequate detection. If averages for small fish are compared with individual measurements for larger fish, a question arises as to the valid measurement of statistical precision. By the season, comparisons of equations based on totals with those based on averages of Y and X_1 (Tables 1 and 2), indicate that equations based on averages are "better" with respect to the statistical significance of parameters. However illusory the statistical advantages of the choice may be, Equations 6, 7, 8, and 10 are selected as best representing seasonal relationships among the variables.

LIMNOLOGICAL CONSIDERATIONS

The choice and interpretations of Equations 6, 7, 8, and 10 of Tables 1 and 2 become simplified if the limnological data of

Figure 1 and the seasonal changes in the behavior of bluegills are considered together. These equations apply only within the range of tabulated values in the Appendix Tables A–D, and they apply to "population averages" under natural conditions in contrast to most published information on laboratory acclimated fish.

Seasonal delimitations

For the 1956–57 period, the utilization of water temperature change or stability as criteria for seasonal grouping of the data is not altogether arbitrary, since changes in water level, abundance of aquatic invertebrate food supply, and condition of the fish also corresponded closely to the thermal characteristics. Since little is known of seasonal changes in respiratory or metabolic rates of fishes, it is thus premature to use changes in these rates themselves as criteria for delimitation of "physiological seasons." Seasonal variation in respiration has been reported by Wells (1935), and endocrine changes associated with gonadal development and spawning, which is seasonal for the bluegill, would undoubtedly influence metabolism (see review by Hoar 1957). Although some of the individual partial regression coefficients do change from season to season (Table 1), usefulness of the seasonal breakdown is evident from the relatively high multiple correlations (Table 2), which would not occur if there were large intraseasonal variations in respiratory rates.

Effects of weight

The partial regressions b_1 of Equations 6, 8, and 10 relating log oxygen consumption rate to log weight are remarkably close to the standard values of *ca.* 0.85 given by Krogh and many later workers. Scholander *et al.* (1953) observed approximately the same value for a wide variety of tropical and arctic poikilotherms. The summer value of 1.06 (Equation 7) is high, quite possibly because several of the larger fish were in spawning condition and might have higher metabolic rates than the smaller fish which had passed their spawning season (Fry 1957, Hoar 1957). For future studies the feasibility of adding another independent variable, such as gonad weight, to provide a measure of sexual development should be investigated.

There are many arguments for expressing weights on a nitrogen or protein weight basis (Gerking 1952, 1954, 1955; Prosser *et al.* 1950; Zeuthen 1947, 1953), especially for the purpose of reducing variability due to wide seasonal variations in fat content. Since the respiratory quotient for fat is lower than for protein, the respiratory rate of a fat fish would be lower than for a lean one, all other conditions being equal (Prosser *et al.* 1950). In this study, however, the magnitude of seasonal variations in respiratory rates is perhaps better clarified by the use of total weights which reflect seasonal variations associated with growth conditions.

Effects of swimming velocity

The relationships of the log oxygen consumption rate to swimming velocity (b_2 in Equations 6, 7, and 8) are approximately the same, considering the fairly large standard errors involved. For these equations, the spring, summer, and autumn average velocities are 3.43, 9.06, and 6.85 meters per minute corresponding to respective average temperatures of 19.51°, 24.07°, and 15.30°C. No attempt was made to calculate a winter average velocity due to "spontaneous" activity of the non-swimming fish. Direct observations from spring through autumn indicate that activity of the fish follows this same pattern. The spring increase in activity, while coinciding with rising temperatures, may also be influenced by the increased output of gonad stimulating hormones. Hoar *et al.* (1955) note that this phenomenon can increase swimming activity and thereby increase oxygen consumption rates instead of increasing metabolism directly. The apparently high rate of swimming in the autumn is a real phenomenon possibly associated with increased efforts in seeking for the dwindling food supply. Although swimming velocity varies considerably over the spring-autumn period, the fact that the partial regressions coefficients do not is a point of considerable interest, inasmuch as the same level of respiration with respect to swimming

movements is implied over this period. The high coefficient for the winter period, however, may mean that movement can be accomplished only at the expense of a much higher relative respiratory rate. Job's (1955) data for *Salvelinus fontinalis* show that the standard rate as a percentage of the active rate of oxygen consumption at 5°C is considerably less than the corresponding percentage for temperatures up to about 14°C. If Job's active rates are for similar swimming velocities at these two temperatures, then the regression of oxygen consumption on swimming velocity from standard conditions to a given active condition would also have to be greater at the lower temperatures.

Effects of temperature

From the considerable literature on temperature acclimation and preferenda it would appear that the partial regression coefficients b_3 would vary so much that they could be statistically significant only if the bluegills were actually acclimated to the temperatures at which the respiratory rates were determined. This condition appears to be generally realized except for the spring data. Since the March data are inadequate because of wide temperature fluctuations from hour to hour, it is important to examine also the temperature conditions during the April–June period for which the temperature coefficient 0.0069 is not significant and is probably too low. During this period bluegills will remain over the shallow spawning beds in spite of wide day-to-day or hour-to-hour temperature variations. Unless there is adequate wind-driven circulation of surface waters, these shallow waters can vary an estimated 5°C diurnally from hot calm days to cool nights. That spawning fish actually confine themselves to the spawning beds during such fluctuations is evident from observations during May and June, 1955, when there was an extensive kill due to oxygen depletion in the inshore areas of the lake. At this time only spawning bluegills were killed; the non-spawning largemouth bass and bluegills were presumably neither killed nor confined to the areas of temporary oxygen depletion and wide temperature fluctuation. Thus there is good reason to believe that spawning bluegills during the spring period are not always thermally acclimated in the classical sense of laboratory determinations.

From a comparison of Equations 6, 7, 8, and 10 in Tables 1 and 2, it appears that the log oxygen consumption rate-temperature regressions are definitely greater during the summer and winter. Fry (1957) discusses this phenomenon in terms of routine and active metabolism for *Salvelinus fontinalis* acclimated to various temperatures. Job's (1955) and Sullivan's (1954) data for this species show that oxygen consumption rates can be relatively higher at temperature extremes. Fry believes that the physiological basis for this condition at temperature extremes is possibly due to increases in spontaneous movements which are not reflected in increased swimming velocities. Irritability and an increase in spontaneous movements mentioned above did occur in the winter but were not especially noticeable during the summer field work at temperatures of about 23–26°C. A high level of spontaneous movements did occur, however, for bluegills in an aquarium at about 30°C.

Effects of sex

There is little in the literature to indicate that sex of fish has an appreciable effect on metabolism. However, much of the experimental work applies to sexually immature fish. For many larger fish in the laboratory there would also not be expected the usual seasonal sexual cycle that occurs in nature. From the inconclusive results of this study, it would seem that any effect of sex on metabolism could be established in the field only by confining experiments to more limited environmental fluctuations.

Effects of grouping

The phenomenon of "social facilitation" for animals including fishes is generally recognized (Allee *et al.* 1949) at least on the basis of laboratory experimentation. From the field study of bluegills, however, it would appear that the respiratory rates for groups are not correspondingly lower than

the rates for single fish in all seasons. The groups of bluegills during the spring and the one group in the summer that contained sexually mature specimens had higher than average oxygen consumption rates. The other three summer groups consisting of immature or post-spawning fish had lower than average oxygen consumption rates as would be expected on the basis of goldfish experiments by Schuett (1934), Escobar *et al.* (1936), and Shlaifer (1938, 1939). Pritchard (personal communication) found that groups of bluegills in late summer consumed oxygen per unit weight at significantly lower rates than did single fish, while in winter there was no difference in the rates between groups and individuals. Direct observations of bluegills and information from seining indicated that both spawning and non-spawning fish of all sizes largely confined themselves to shallow waters during the spring and early summer in concentrations 10 to 100 times denser than in late summer and autumn when the fish tended to disperse. Earlier in the season the fish were wary and antagonistic toward each other; later, when in loose schools and more widely distributed, they appeared much more placid. Both Schuett and Shlaifer noted that the numbers in the groups within a given volume would influence the rate of oxygen consumption but not in a straightforward inverse relationship. Shlaifer (1939) found that vision was directly involved. While the results from the bluegill experiments would appear to agree with direct observations under natural conditions, the results are insufficient to indicate effects of varying numbers and the possibilities of acclimating the spring and early summer fish within the chamber so that they would exhibit social facilitation rather than antagonism.

SEASONAL EFFECTS ON BIOLOGICAL PRODUCTIVITY

Gerking (1954) has shown how data for bluegill metabolism and population dynamics can be combined for an estimate of the total protein turnover in a population. He defined protein turnover as the amount of protein that is required by the population for growth, for the metabolic expenditure of energy, and for the replacement of proteins broken down during the normal course of living. Although the weight data in this study are expressed as live weight, and not as protein weight, Gerking's concepts can be applied at least qualitatively to an interpretation of Equations 6, 7, 8, and 10 (Table 1) with respect to limnological variables affecting the Felt Lake bluegill population.

In a general way the seasonal trend of metabolic rates varies with the temperature (Fig. 1). Yet in the spring and early summer the fish not only grow rapidly and get fatter, but their gonads also develop rapidly in contrast to the autumn period at similar temperatures when the fish tend to lose weight. Since the obvious cause of the weight decline in autumn and winter is lack of food, it would seem that the population could maintain weight at this time if metabolic "efficiency" increased, if metabolic rates dropped, or if the population decreased so that competition for food was eliminated. Only the last condition appears to be even partially realized, and there is no evidence to indicate that adverse autumn and winter conditions reduce the reproductive potential in the spring. If there were any kind of "metabolic adaptation" (akin to hibernation or aestivation) to these seasonal extremes, it would seem that the metabolic rates would thus change markedly from spring through autumn and winter.

A comparison of Equations 6 and 8 for an intermediate spring or autumn temperature of, say, 17°C for observed weights and activities yields expected respiratory (or metabolic) rates of the same order for the two periods. Furthermore, the expected rates, calculated from Equations 6, 8, and 10 over pertinent weight and activity ranges for a winter temperature of, say, 10°C, are also of comparable magnitude. There thus is no evidence from these equations that the respiratory or metabolic rates decline during the adverse growing seasons much more than would be expected solely on the basis of the decline in temperature, and possibly activity. There is,

however, some physiological basis for the apparent lack of metabolic adaptation to poor food conditions.

In the spring the addition of fat can be explained not only by the increased food supplies and the greater feeding intensities, but also by the fact that at increased feeding rates there tends to be an increase in fat content (Gerking 1955). Gerking (1954) reported that fat content could rise to about 33 per cent for northern Indiana bluegills. On this basis and on the basis that the respiratory quotients for carbohydrate, protein, and fat are respectively 1.00, 0.79, and 0.71 (Prosser *et al.* 1950), adjustment of the spring rates for fat fish (Equation 6) to a protein basis would be but slightly upward. In a similar manner the increased plant consumption (and carbohydrate intake) in the autumn and winter might be responsible for higher rates. However, there appears no conclusive evidence that carbohydrate is actually digested, although the herbivorous propensities of the bluegill during food shortages have been widely reported (Ball 1948, among others). Gerking (1952) also noted that while two centrarchids absorbed protein efficiently at all ages, younger fish utilized protein for growth more efficiently than did older fish. Inasmuch as most all of the fish in this study were a full growing season older in the autumn and winter than in the spring, this "aging" phenomenon would also account for higher rates by the end of the growing season. When Equation 10 for winter is used to estimate respiratory rates at spring or autumn temperatures, it is evident that the comparably high rates are probably explained by "cold adaptation" as defined by Scholander *et al.* (1953).

With aging, with cold adaptation, and with the increasing carbohydrate consumption along with a poorer protein diet and declining fat reserves, it is likely that the metabolic rates of bluegills on a live weight basis would tend to increase during autumn and winter, other factors being constant.

From the standpoint of biological productivity, it may be noted that the annual growth rates of Felt Lake bluegills are estimated to be of the same order as those reported for northern Indiana bluegills 90 millimeters and longer by Gerking (1954). From Figure 1 data, it appears that the Felt Lake growing season (at temperatures above 15–16°C, say), during which the environment is reduced to about half its maximum with an accompanying decrease in the production of bottom fauna, is *potentially* at least twice as long as in northern Indiana. Allowing for the inherent dangers in basing calculations on variously derived data, a generalized comparison of Gerking's (1954) Gordy Lake, Indiana, protein turnover data with calculated values from Felt Lake is possible assuming that the annual growth of the larger bluegills in these diverse environments is of the same order. This assumption is justified to the extent that Felt Lake bluegills from ages I to IV have instantaneous growth rates roughly equivalent to those of ages II to V in Gordy Lake (Gerking 1954, Fig. 2). While Felt Lake bluegills grow as much in their first year as Gordy Lake bluegills grow in their first two years, the poor feeding conditions and the high mortality rates make survival and growth rates beyond age IV very poor. Hence the average instantaneous growth rate in each population would be of the same order.

For an average Gordy Lake summer population of 8979 fish weighing 420.0 kg the average weight of a fish would be 46.78 g. By interpolation from Gerking's (1954) Table I and Figure 3, a fish of this size would require about 19 g protein for seasonal growth at a 31 per cent efficiency of protein utilization for growth alone. On this basis, for growth, metabolic energy expenditure, and protein replacement the total seasonal protein requirement would be about 61 g for a 46.78 g fish. Since there are, crudely, 5.65 Cal/g protein and 1 L oxygen consumed in generating 4.82 Cal upon the oxidation of mixed protein (Brody 1945), there are consumed 1.172 L oxygen per gram of oxidized protein. Thus the oxidation of 61 g protein per fish would require about 71 L oxygen. Since Gerking's growth experiments with presumably inactive fish at a temperature of 25.9°C indicated that 150 days would be

required to account for the seasonal growth, a direct comparison with Equation 7 (Table 1) for Felt Lake is possible. From this equation the log mg oxygen uptake per hour for a 46.78 g fish at 25.9°C and zero velocity would be 1.0472, or 11.14 mg/hr, or 7.803 cc/hr. At the average summer swimming velocity of 9.06 m/min in Felt Lake, the oxygen consumption would be increased to 10.41 cc/hr. In 150 days 28 L oxygen would be consumed at zero velocity or 38 L at the average observed velocity. Both of these Felt Lake values are much lower than the 71 L calculated for Gordy Lake and indicate that Felt Lake bluegills with a long potential growing season have a much lower production rate per unit weight of fish.

Therefore, without apparent mechanisms for suppressing maintenance metabolic requirements after the growing season or for maintaining adequate food supplies to allow for potential growth over the entire growing seaon, the biological productivity of the bluegill in western U. S. waters of the Felt Lake type would be necessarily poor compared to the productivity attained in its native eastern U. S. habitats.

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APPENDIX

Felt Lake bluegill data tabulations by seasons. Code for Tables A to D.

Column 1—Date of experiment.

Column 2—Experiment number.

Column 3—Log milligrams oxygen consumed per hour.

Column 4—Log weight of fish in grams.

Column 5—Activity of fish (meters per minute).

Column 6—Temperature of water (°C).

Column 7—Log milligrams oxygen consumed per hour per kilogram.

Column 8—Sex: M, male; F, female; or number of small fishes (usually immature).

APPENDIX TABLE A: *March–June Data*

(1)	(2)	Y (3)	X_1 (4)	X_2 (5)	X_3 (6)	Y_1 (7)	(8)
12 III 56	1	1.493	2.511	0.0	14.8	1.982	4
	2	1.534	2.339	7.8	17.8	2.197	3
	3	1.533	2.210	10.1	17.8	2.322	2
	4	1.312	2.072	12.0	17.0	2.240	2
24 III 56	5	1.292	2.137	1.5	17.6	2.155	2
	6	1.316	1.924	5.5	15.5	2.393	2
27 III 56	9	1.330	2.225	1.5	16.4	2.104	3
	10	1.008	2.025	0.0	15.0	1.983	F
7 IV 56	11	1.143	1.973	1.5	17.7	2.170	5
	12	1.127	1.954	0.0	19.3	2.173	M
	13	1.112	1.892	1.1	18.5	2.219	M
	14	1.624	1.924	7.7	18.5	2.700	M
14 IV 56	15	1.234	2.045	6.0	14.0	2.189	F
	16	1.131	2.021	6.0	14.9	2.109	F
	17	1.148	1.954	1.0	14.9	2.194	M
21 IV 56	18	1.312	2.072	0.5	19.7	2.240	5
	19	1.258	2.004	0.0	20.5	2.254	8
	20	1.332	2.065	0.0	21.9	2.269	5
5 V 56	21	1.022	1.881	8.5	18.8	2.141	M
	22	0.946	1.944	0.0	19.0	2.001	F
	23	1.277	2.124	0.0	19.0	2.154	5
12 V 56	24	1.229	1.982	1.1	17.0	2.247	1
	25	1.206	1.954	6.7	21.0	2.252	F
19 V 56	26	1.088	1.863	1.2	20.0	2.224	8
	27	1.034	1.903	0.6	20.0	2.131	7
	28	1.352	2.076	0.0	21.0	2.276	6
2 VI 56	29	1.336	2.000	5.8	21.5	2.336	3
	30	1.241	2.009	0.0	23.0	2.233	2
23 VI 56	31	0.925	1.924	8.5	22.8	2.000	M
	32	1.452	1.978	12.5	22.8	2.474	F
	33	1.511	1.982	10.3	23.0	2.529	F

APPENDIX TABLE B: *July–August Data*

(1)	(2)	Y (3)	X_1 (4)	X_2 (5)	X_3 (6)	Y_1 (7)	(8)
7 VII 56	34	1.454	2.100	8.4	23.5	2.353	F
	35	1.060	1.845	1.0	24.2	2.214	M
	36	1.352	1.833	1.0	24.5	2.519	M
13 VII 56	37	1.468	1.991	12.4	23.1	2.477	F
	38	1.426	1.991	14.4	23.2	2.435	F
21 VII 56	39	1.393	1.914	9.7	24.5	2.480	M
	40	1.324	1.973	3.0	24.5	2.351	F
	41	1.478	1.886	11.5	25.8	2.592	F
28 VII 56	42	1.491	2.004	13.0	25.0	2.487	F
	43	1.461	1.964	12.3	25.2	2.497	F
	44	1.428	1.881	12.0	25.2	2.547	F
	45	1.490	2.017	11.7	26.0	2.473	F
	46	1.549	2.000	6.7	26.5	2.549	F
14 VIII 56	47	1.075	1.748	9.3	25.0	2.327	10
	48	1.481	2.083	9.7	25.0	2.398	18
	49	1.268	1.875	8.7	24.0	2.393	5
	50	1.300	1.908	9.3	22.9	2.391	3
15 VIII 56	51	1.260	1.924	9.3	22.0	2.336	F
	52	0.868	1.785	8.3	22.2	2.083	M
	53	1.327	1.792	11.7	22.2	2.535	F
	54	1.224	2.025	7.0	22.5	2.199	M
	55	1.103	1.833	9.0	22.5	2.270	M

APPENDIX TABLE C: *September–December Data*

(1)	(2)	Y (3)	X_1 (4)	X_2 (5)	X_3 (6)	Y_1 (7)	(8)
17 IX 56	56	1.353	1.806	5.3	21.5	2.547	8
	57	1.105	1.602	0.0	22.0	2.503	9
	58	1.299	1.919	14.3	22.0	2.380	F
	59	1.294	1.935	13.3	22.0	2.360	F
	60	1.264	1.778	11.3	22.4	2.486	M

APPENDIX TABLE C—*Continued*

(1)	(2)	Y (3)	X_1 (4)	X_2 (5)	X_3 (6)	Y_1 (7)	(8)
18 IX 56	61	1.108	1.813	8.3	21.3	2.295	M
	62	0.935	1.833	10.7	21.5	2.102	F
	63	1.229	1.820	11.3	22.0	2.410	1
20 X 56	64	1.192	1.924	7.3	18.0	2.268	11
	65	1.268	1.869	9.7	18.2	2.399	2
	67	1.198	1.833	6.7	19.0	2.366	F
	68	1.234	1.935	10.3	19.1	2.300	M
	69	1.100	1.973	0.0	19.6	2.127	2
3 XI 56	70	0.960	1.892	0.0	14.9	2.068	F
	71	1.250	1.863	6.0	15.0	2.387	M
	72	0.938	1.792	1.3	15.5	2.146	F
17 XI 56	74	1.057	1.839	10.7	13.9	2.218	F
	75	1.299	1.833	10.7	13.9	2.466	M
	76	0.989	1.851	9.0	14.0	2.138	2
	77	1.341	1.820	7.3	13.9	2.521	M
	78	1.108	2.000	2.0	13.9	2.108	2
23 XI 56	79	0.831	1.813	11.3	12.0	2.019	F
	80	0.944	1.756	10.0	12.0	2.189	F
	81	0.921	1.820	12.3	12.5	2.102	M
	82	1.117	1.799	5.7	12.5	2.317	F
	83	0.720	1.748	8.7	12.5	1.972	M
1 XII 56	84	1.046	1.973	7.0	11.0	2.073	F
	85	1.018	1.929	4.3	11.1	2.089	M
	86	0.555	1.845	0.3	11.9	1.709	M
	87	0.995	1.839	6.0	12.5	2.156	M
	88	1.109	1.845	8.0	12.5	2.264	M
	89	1.104	1.869	6.3	12.0	2.235	F
13 XII 56	90	1.207	1.924	10.7	11.7	2.283	F
	91	1.046	1.898	4.3	12.0	2.148	M
	92	1.032	2.017	0.3	12.3	2.015	M
	93	1.163	1.903	0.0	12.3	2.260	F
14 XII 56	94	0.427	1.826	2.8	11.4	1.601	M
	95	1.157	1.973	5.3	12.0	2.184	2
	96	1.118	2.097	7.0	12.1	2.021	2
	97	1.166	1.869	8.2	12.2	2.297	F

APPENDIX TABLE D: *January–February Data*

(1)	(2)	Y (3)	X_1 (4)	X_2 (5)	X_3 (6)	Y_1 (7)	(8)
12 I 57	98	0.995	1.881	9.0	8.2	2.114	M
	99	1.097	1.826	0.0	8.4	2.271	F
16 I 57	100	0.795	1.845	5.3	9.0	1.950	M
	101	1.131	2.076	1.7	9.1	2.055	F
	102	1.095	1.785	6.3	10.0	2.310	F
	103	0.938	1.813	0.7	10.0	2.126	F
	104	0.964	1.869	4.3	10.0	2.094	M
	105	0.977	1.806	4.5	9.5	2.170	M
25 I 57	106	0.953	1.881	6.7	8.5	2.072	F
	107	0.630	1.716	2.7	8.5	1.914	2
	108	0.663	1.732	5.0	8.4	1.930	M
	109	0.940	1.699	6.2	8.5	2.241	F
9 II 57	110	1.057	2.025	1.7	9.2	2.031	F
	111	1.073	1.833	0.0	9.2	2.241	M
	112	0.806	1.792	0.0	9.4	2.013	M
22 II 57	113	1.125	1.863	0.7	11.9	2.262	M
	114	1.082	1.833	0.8	12.0	2.249	F
	115	0.638	1.806	0.0	12.0	1.832	F
	116	1.162	1.875	0.0	11.9	2.287	F
	117	0.985	1.903	0.5	11.9	2.082	M
	118	1.141	1.857	0.0	11.9	2.284	F
	119	0.899	1.748	0.0	11.9	2.150	M

Polarographic Oxygen Electrode[1]

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ABSTRACT

A method has been developed for the continuous monitoring of oxygen tension in solutions and gases. The oxygen is reduced at a platinum surface which is covered with a plastic membrane. The silver-silver oxide reference electrode is also included under the membrane. Drift is less than 10 per cent for a period of days, and temperature compensation is possible. A number of uses to which it has been put are described.

The intimate involvement of oxygen in nature makes its measurement a problem of recurring interest. In many investigations present techniques of oxygen determination limit the amount and type of data that can be taken. A method of continuously monitoring oxygen tension in liquids and gases will be described in this paper. It is based on the polarographic reduction of molecular oxygen at a platinum electrode. Many of the previous shortcomings of polarographic systems have been overcome. The variety of tasks to which it has been applied include the determination of biological oxygen demand in domestic sewerage, continuous flow respirometry of a variety of marine and freshwater invertebrates and fishes, determination of respiration and photosynthesis in algae, analyzing respiratory gases for oxygen, determination of oxygen tension in 1-ml samples of water and biological fluids, and vertical profiles of oxygen in fresh and salt water. Interest has been sufficient to indicate that the technique should be published separately as a research method. An explanation of how the electrode functions will be followed by a description of some of the above uses to indicate its potentialities. A more complete description of the theory and chemical behavior of the electrode will be presented elsewhere.

I. Theory of the Oxygen Electrode

The present system consists of a platinum electrode held at −.8 volts to a silver-silver oxide reference electrode. Molecular oxygen is reduced at the platinum surface, and a current flows. The magnitude of this current depends upon the amount of oxygen reaching the platinum surface. Thus a bare platinum electrode in sea water makes an acceptable polarograph for a short time. The sensitivity of such an electrode decreases with time, apparently due to metals plating out on the platinum. The sensitivity cannot be regained by reversing the current. In complex biological media such as blood, red cells or other material usually collect on the surface and reduce the sensitivity by partially blocking the access of oxygen. Dropping mercury electrodes partly overcome the difficulty by continually renewing the metallic surface, but they are inconvenient and may be poisonous to living systems. Such chemical and physical factors have prevented polarographic methods of oxygen determination from being widely used.

In an effort to overcome the shortcomings of a bare platinum electrode in biological fluids, Davies and Brink (1942) covered the metal with agar and also a membrane of collodion, and Clark and colleagues (1953) later investigated a variety of other plastic films. A recent symposium has summarized the present state of the art (Federation Proceeding 1957). Interest in such systems has been mostly among biologists for application to specific problems. The present work was undertaken to develop a generally useful technique for biological and oceanographic work.

Preliminary experiments with uncovered

[1] Contribution No. 1014 from the Woods Hole Oceanographic Institution.

electrodes led to the conclusion that a practical platinum polarographic system must have two features. The platinum should operate in a simple medium to prevent changes in electrode sensitivity due to chemical interference, and there should also be a standardized diffusion arrangement between the platinum surface and the medium in which oxygen is being measured. Polyethylene and teflon have the necessary properties to realize both of these conditions. Molecular oxygen can diffuse through them, while at the same time they are impermeable to ions. The platinum electrode can operate behind a thin film of polyethylene in a pure solution of KOH. Under this condition its sensitivity does not change. The plastic represents a considerable but fortunately unchanging diffusion barrier.

The physical arrangement of the electrode system is shown in Figure 1. The silver-silver oxide reference electrode is concentric around the platinum. A film of polyethylene is stretched tightly over this and held by a retaining ring. The film is put on the electrode while the entire assembly is held under the surface of a solution of 0.5 N KOH. A small amount of this solution is trapped under the plastic. It forms only a very thin layer between the plastic and the platinum.

The initial reaction of the oxygen at the platinum forms OH ions.

$$4e^- + O_2 + 2H_2O \rightarrow 4OH^- \qquad (1)$$

A piece of indicator paper laid across the platinum under the plastic shows increased pH a few seconds after a current due to O_2 starts to flow. The OH ions then diffuse to the reference electrode and react with the Ag to form Ag_2O.

$$2Ag + 2OH^- \rightarrow Ag_2O + H_2O + 2e^- \qquad (2)$$

The end result is the formation of Ag_2O which appears as a dark brown or black coating on the surface of the silver. The Ag_2O then forms the half cell which makes contact with the solution to complete the circuit with the platinum electrode. Since the Ag can be made thick such an electrode

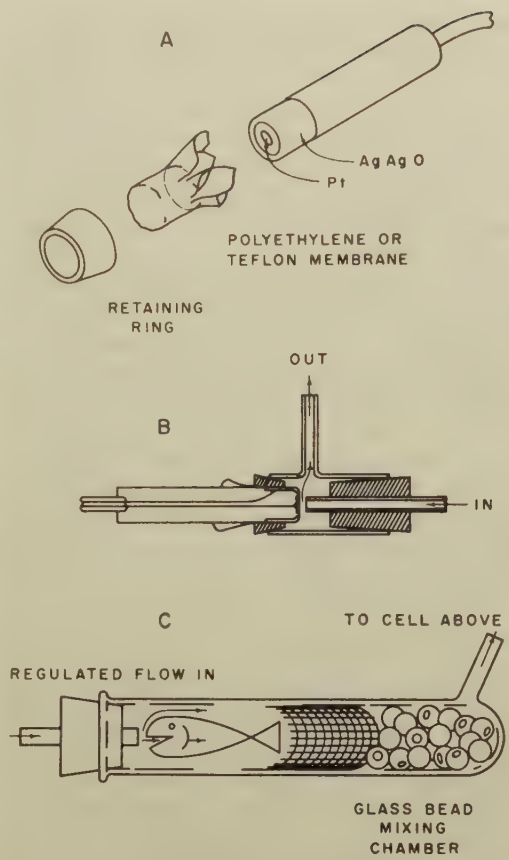

FIG. 1. *A*. Exploded view of the oxygen electrode showing how the membrane is stretched tightly over the end surface and held this way by an elastic retaining ring. *B*. Cell with which the electrode can be introduced into a stream of solution or gas. *C*. An arrangement for continuous flow respirometry of small fishes or other aquatic animals.

system will last for many months of continuous operation.

The diffusion situation of O_2 in the electrode is as follows. When the electrode is turned on a current passes which is the same as if a bare electrode were used, since the KOH solution has previously established oxygen tension equilibrium across the film. The current decreases in a few seconds as the small amount of oxygen in solution behind the film is consumed by the above reaction. Oxygen from the medium begins to diffuse across the film because of the gradient produced. A steady state is soon reached where the oxygen consumed at the

platinum equals that diffusing across the film. With a 0.0015″ thick polyethylene film the current will now be about $\frac{1}{1000}$ as large as if the platinum were uncovered. The O_2 tension behind the film must be reduced from that outside by this factor. Thus in air-saturated water this tension is only a fraction of a mm of Hg when a steady state is reached.

If the electrode is turned off and then turned on again, the current is initially very large. Oxygen tension equilibrium has been partly reestablished across the film in the interim. If a sensitive meter is used as a current indicator, it is necessary to protect it from this initial surge of current.

The tension increment across the film is nearly equal to the value of the tension in the medium, since it has been shown that that in the KOH is very low. So little oxygen diffuses from the medium into the film the system is much less dependent upon the flow of water past the electrode. Going from an absolutely quiescent condition to vigorous stirring increases the current only 25 to 50 per cent. A very slight motion of about 1 cm/sec. brings the current nearly up to saturation. This much movement is supplied easily by random motion when an electrode is dangled on the end of a cable over the side of a boat.

The platinum electrode "sees" more than its own area of polyethylene, and diffusion takes place through this increased area. Due to this edge effect the polarographic current does not increase linearly with the area of platinum. For electrodes of less than 2 mm diameter, it is more nearly a function of the diameter. This gradually changes to an area dependence in larger electrodes as the edge area becomes a smaller fraction of the whole. Platinum electrodes from 0.2 to 2 cm in diameter have been used. There seems to be no reason why larger electrodes with correspondingly greater currents are not feasible.

Equation 1 states that the electrode reaction consumes a molecule of oxygen for every 4 electrons of current flow. If one microamp of current flows for one hour the amount of oxygen consumed is about 2×10^{-4} ml. This consideration may limit the current and thus the size of the electrode to be used in a small closed system, where the total quantity of dissolved oxygen is small.

Most of the electrode systems have been made from a length of silver tubing and a disc of platinum. These are soldered onto a cable and then cast in an epoxy plastic so that only the two desired metals are exposed. Lucite has also been used successfully as an insulating support for the electrodes.

The silver is then electrolytically coated with oxide until it is black. This eliminates a period of several hours initial equilibration. The area of the silver should be at least ten times that of the platinum for the quickest response.

The polarographic cell is an amperometric device in which the oxygen concentration rather than the voltage applied determines the current that flows. With a given oxygen concentration this current increases about 5 per cent for every degree centigrade. Unless measurements are made at the calibration temperature, they must be corrected. Automatic compensation can be effected by taking the voltage drop of the current through a thermistor with a temperature coefficient equal in magnitude and opposite in sign to the cell. The thermistor is exposed to the same temperature as the solution being measured and can conveniently be cast in the plastic electrode block. The relatively high temperature coefficient represents a combination of the effect of temperature on the diffusion and solubility of oxygen, and also the temperature kinetics of the chemical reactions involved.

A 1-cm platinum electrode covered with a 0.0015″ polyethylene film will give a current of about 20 microamperes at 25°. This can be measured directly on a meter. For smaller electrodes it is necessary to use either a galvanometer or recording potentiometer. The latter instrument can be driven from the voltage drop across a ten-turn potentiometer. With provision for offsetting the zero, the recorder can be made to cover only the range of interest.

If small changes in oxygen are to be recorded, the flow past the electrode must

be constant. To accomplish this in closed bottles, magnetic stirring has been used. A large magnetron magnet turned by a synchronous motor will rotate a small magnet inside a bottle in a water bath from as far as a foot away.

Small fluctuations in cell current have been traced to small bubbles on the polyethylene. Unfortunately bubbles seem to form preferentially on plastic rather than glass. The trouble usually develops when the water being measured has been warmed up from a lower temperature, and is thereby super-saturated. For coarse work a few small bubbles do not seem to matter.

A cell for continuous flow work is shown in Figure 1B. The entering flow is directed against the area of plastic directly over the platinum. By bringing the entering tube to within 0.5 mm of the electrode surface, a flow of 5 to 10 ml/min. can produce a saturation current.

It must be kept in mind that a polarographic electrode is a diffusion-dependent device and therefore measures oxygen tension rather than content. Fresh and salt water equilibrated with the same gas mixture will give the same reading despite the salt water having less oxygen. If an instrument is to read in amount rather than in tension, it must be calibrated with a solution of the same gas solubility. Fortunately the salting out effect on gas solubility is not large. A change of 0 to 3.5 per cent salt decreases the oxygen solubility by only 20 per cent. When solutions of blood pigments are involved the oxygen content is not linearly related to tension. Reference must then be made to the loading curve of the pigment for the pH and temperature of the measurement. Since tension is the driving force of diffusion, it is sometimes a more pertinent variable than oxygen content.

The overall analytical performance of these electrodes has been investigated with a recording potentiometer and independent physical determinations of the oxygen tension (Scholander *et al.* 1955). Since these latter measurements were accurate only to 1 or 2 per cent, knowledge of the electrode is correspondingly limited. Operation of the device is as follows:

1. Current is a linear function of oxygen tension over at least the range of 0 to 1 atmosphere oxygen.

2. It is stable to a few per cent over a period of days, and changes less than 25 per cent in a period of several weeks. During the time there are no abrupt changes. Thus, standardization every few hours is sufficient.

3. Short period changes of 0.01 ml per liter in sea water can be detected. This is about 0.2 per cent of the amount in solution when equilibrated with air.

4. The electrode as described gives 90 per cent of full response in about 20 seconds, and has reached 99 per cent in 2 minutes.

5. The electrode has operated successfully in dilute acids and alkalies, sea water, and a variety of polluted waters. No chemical interference has been noted. Only gross fouling on the plastic surface appreciably reduces the current. It is insensitive to the osmotic concentration of the medium.

II. EXPERIMENTAL USES

A. Algal respiration and photosynthesis

The algal sample is placed in a bottle of sea water with a stirring magnet. An

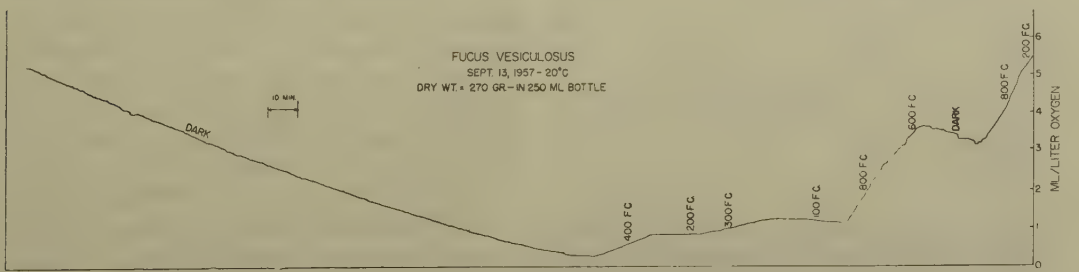

FIG. 2. Continuous time curve of oxygen in a closed bottle containing algae. The change in slope represents different rates of respiration and photosynthesis.

oxygen electrode is used as the stopper. Changes in dissolved oxygen are recorded while the alga is exposed to different light intensities in a temperature bath. The resulting curve for *Fucus vesiculosus* in Figure 2 shows an initial decrease of oxygen in the dark due to respiration. The oxygen tension dependence of respiration is shown by the decrease in slope as the oxygen content becomes smaller. This is plotted in Figure 3. The drop is probably due to diffusion limiting the supply of oxygen to the cells at the center of the relatively thick thallus. Thin species such as *Ulva* maintain a nearly constant respiratory rate to lower values of oxygen.

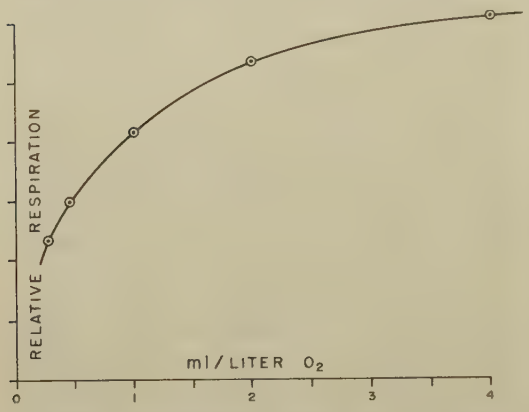

FIG. 3. Curve showing variation in respiration with oxygen tension in Figure 2.

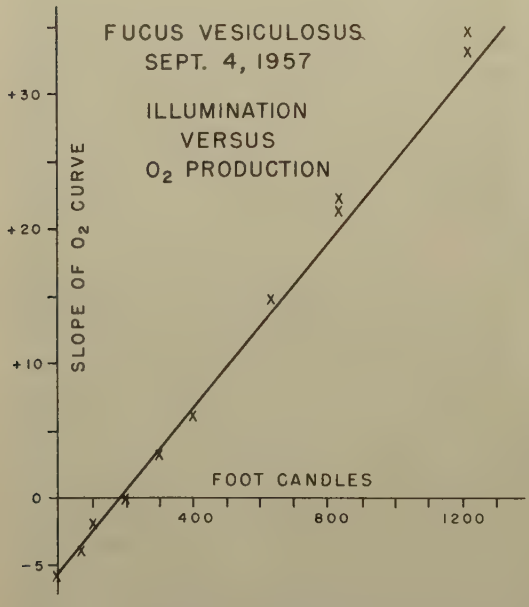

FIG. 4. Change of slope with illumination in Figure 2. This is a measure of the rate of oxygen production and therefore of photosynthesis.

The rates of oxygen production at different light levels are indicated by changes in slope from that in the dark. The slope was zero at 200 foot-candles, indicating that photosynthesis just balanced respiration. In Figure 4 it is clear that at 1200 foot-candles there was no sign of saturation. Quantum efficiency studies in the ultra violet are being done in an analogous manner with a quartz chamber.

C^{14} labelled carbon dioxide is being used to follow carbon fixation simultaneously with oxygen production during photosynthesis in unicellular algal cultures. The culture is confined in a hypodermic syringe with an electrode in the end of the plunger. With such a container serial samples can be withdrawn for counting while the oxygen in solution is continuously recorded.

B. Animal respirometry

The oxygen consumption of many invertebrates and fishes has also been measured in closed containers. If the animal is allowed to remove all the oxygen, an analogous curve relating respiration to oxygen tension can be calculated. The electrode has also been used in a continuous flow respirometer in which the water in the animal chamber is continously replaced. The animal is thus not exposed to an increasing accumulation of its own waste products. Flow rate times the increment of oxygen between ingoing and outgoing water indicates the instantaneous respiration rate. The time resolution of such a system depends on how fast the water in the animal chamber is replaced, and thus is a function of flow rate and chamber volume. If the flow rate and oxygen content of the entering water are constant, a single electrode in the water leaving the chamber will record the time course of metabolism. The method has been useful with a variety of animals ranging from a 0.5-g snail to a 30-kg shark.

An arrangement for measuring the metabolism of a fish weighing a few grams or more is shown in Figure 1C. The fish is placed in a tube small enough so that it cannot turn around. Its head faces the incoming water. Some of the water goes into the fishes mouth and through its gills. The rest passes around the fish. These are mixed in the glass beaded chamber at the end of the tube, and pass to the electrode. One can see wide excursions in the oxygen uptake when the fish is frightened by a light or a noise. A motor driven syringe has been used to deliver a toxic substance to the inflowing water. The time course and severity of its effect on oxygen consumption could be recorded.

A *Venus* clam was kept continuously in a flow respirometer for several days. As would be expected there was negligible drop in the oxygen of the water leaving the chamber when the shell was closed. Upon opening there was an abrupt decrease as the sea water enclosed in the shell mixed with that in the rest of the chamber. The drop was always the same amount and indicated that the oxygen tension must be close to zero inside the shell when it had been closed for more than 30 minutes. Even when the shell was open with the animal pumping, the instantaneous oxygen consumption varied considerably. An average would have to be taken over a considerable length of time to be an accurate measure of metabolism in such an animal.

C. Small sample techniques

A liquid sample in a syringe can be ejected through the cell in Figure 1B to measure its oxygen tension. A small electric motor has been conveniently used to advance the plunger at a constant rate. A single determination takes two minutes, and needs 5 to 10 ml. Frequent use of calibrating samples make it unnecessary to reach saturation velocity at the electrode surface.

It has also been possible to measure the oxygen tension in a 2-ml sample drawn into a syringe with an electrode in the plunger. A small magnetic rod has been previously placed in the syringe. Stirring for two minutes produces a maximum reading and then a gradual decline as the electrode reaction slowly consumes the dissolved oxygen. It is necessary to handle the sample quickly and with a minimum exposure to the air. A series of standards can be kept in large syringes for running before and after an unknown. Since the tension measurement does not destroy or consume the sample any additional operation can be performed on it afterwards. This technique has been convenient with human blood. After a reading part of the sample can be injected directly into a Van Slyke analyzer to measure oxygen content as opposed to oxygen tension.

D. Field experiments

The electrode has been used as a field instrument to obtain vertical profiles of oxygen in natural waters. Accessory temperature information is necessary to correct the readings. The electrode and a thermistor have both been operated on the same cable. Calibration just before lowering is done by immersing the electrode in a container of water that has been air-saturated by swirling for several minutes.

The hypolimnion and epilimnion clearly showed in a pond used for the local water supply. Water was being drawn from below the thermocline so that tap water was only about one-third saturated in late summer. An electrode was being used to monitor this when a fall storm produced considerable vertical mixing. The tap water oxygen content doubled in two days.

The diurnal oxygen variation in a shallow pond in mid summer was followed by an instrument supported on a pole pushed into the bottom and read by a telescope from shore. A thick plant growth covering the bottom was the main source of photosynthetic oxygen. Bubbles formed on the plants in the sunshine and were analyzed and found to be largely oxygen. The electrode read off scale when pushed in among the plants. The electrode was then hung at mid-depth and moved horizontally from shore by a string to produce a satura-

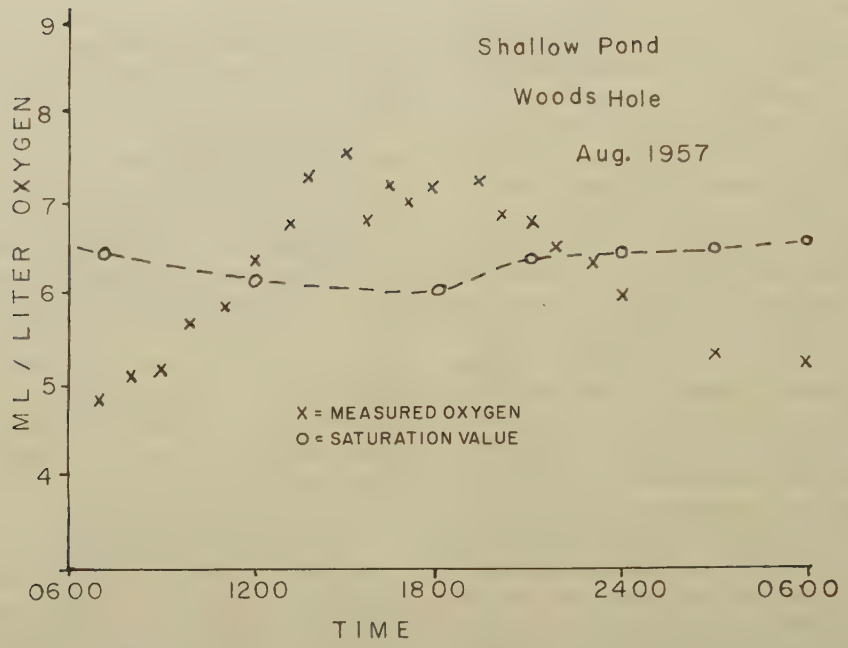

FIG. 5. Diurnal change in dissolved oxygen in a shallow pond is shown by the X's. The saturation value (small circles) changes due to temperature change.

tion current. This movement is only necessary just before taking a reading.

The record for one day in Figure 5 shows oxygen supersaturation produced photosynthetically during the day and a corresponding undersaturation from night time respiration. A strong wind in the afternoon mixed the water and unloaded some of the excess gas. The increasing concentration during the morning can be used to estimate the productivity per unit area when the entire water column is taken into consideration. The uncertain amount of atmospheric exchange makes this a poor method with strong winds.

On one instance a submersible pump was used to deliver a constant stream of water from depths up to 100 feet in the ocean. An electrode was inserted in the line on deck and the pump was gradually lowered. Winkler samples taken at the same time showed that the electrode agreed within 3 per cent. Such an arrangement is only useful to moderate depths in still water. An arrangement for recording in situ with a lowered instrument is being planned.

III. CONCLUSION

Some possible uses of the polarographic oxygen electrode have been explored in the experiments described. Others will be reported in separate publications. As a laboratory instrument the electrode will indicate oxygen tension to 1 or 2 per cent. Because of its marked temperature sensitivity no effort has been made to equal this in field experiments. There seems to be no reason why such accuracy cannot be realized if attention is paid to such factors as flow past the electrode and temperature of the water. The measurement is no more difficult or involved to perform than that of pH.

The development of the electrode has been done in cooperation with Dr. Dayton Carritt of the Chesapeake Bay Institute. He will report more fully on its chemical functioning and on additional uses.

The electrode will be made commercially by the Jarrell-Ash Company, Newtonville, Massachusetts.

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The Use of Conversion Factors for the Determination of the Concentration of Nutrients in Culture Media[1]

Although artificial and enriched culture media have been used for many years, there apparently has been no attempt to standardize the method of reporting the concentration of nutrients in media. The most realistic method of reporting the concentration of elements or compounds is in terms of molar units. However, this unit has not been generally employed in the field of culture study, although oceanographers routinely use the microgram atom unit. Generally, a particular unit of concentration is used by an investigator. It is, though, difficult to translate rapidly from units given by other investigators into terms of one's own experience. It would be desirable to rapidly converting from a concentration of a particular element to the concentration of a compound in which the element is delivered, or vice versa.

Two tables are presented which may be used for rapid conversion into any of the common units desired. In Table 1 an attempt has been made to list the compounds, and the various hydrated forms, generally used as inorganic sources of major elements in culture media. The micro-elements most commonly added to culture media are listed in Table 2, but only the most hydrated compounds are presented. By using these tables, it is possible to rapidly convert from milli-

TABLE 1. *Conversion factors for converting from milligrams of a major element to milligrams of a compound, and from milligrams of a compound to micromoles of a compound*

	N	P	K	Na	Ca	Mg	Cl	S	Si	Micromoles/ milligram of compound
$Na_2HPO_4 \cdot 2H_2O$		5.75		3.87						5.62
$Na_2HPO_4 \cdot 7H_2O$		8.67		5.83						3.73
$Na_2HPO_4 \cdot 12H_2O$		11.56		7.79						2.79
$NaH_2PO_4 \cdot H_2O$		4.46		6.00						7.25
$Na_2SiO_3 \cdot 9H_2O$				6.18					10.15	3.52
$NaNO_3$	6.07			3.70						11.76
Na_2SO_4				3.09				4.43		7.04
NaCl				2.54			1.65			17.11
NH_4NO_3	2.86									12.49
NH_4Cl	3.82						1.51			18.69
$CaCl_2 \cdot 2H_2O$					3.67		2.07			6.80
$CaCl_2 \cdot 6H_2O$					5.47		3.09			4.56
$Ca(NO_3)_2 \cdot 4H_2O$	8.42				5.89					4.23
$MgCl_2 \cdot 6H_2O$						8.36	2.87			4.92
$MgSO_4 \cdot 7H_2O$						10.14		7.69		4.06
KCl			1.91				2.10			13.41
KNO_3	7.22		2.59							9.89
K_2HPO_4		5.62	2.23							5.74
KH_2PO_4		4.39	3.48							7.35

have a table available permitting easy and rapid conversion.

The concentration of nutrients in culture media is most commonly expressed in one of three general ways. The concentration may be expressed in terms of molar units, or as the weight of the element, or of the compound. Concentrations expressed in molar units are easily interconvertible to gram equivalent units. On a weight basis, the concentration may be given as parts per million, milligrams per liter, per cent, milligrams per cent, etc. All of these units are interconvertible, but the difficulty arises in grams of a particular element to milligrams of the compound in which the element is delivered by multiplying the conversion factor times the weight of the element. Conversely, if the weight of the compound is known, the weight of the element can be determined by dividing the factor into the weight of the compound. Factors are also presented in the tables for converting from the weight of the compound to the number of micromoles per milligram of compound. If the weight of the compound in milligrams is known, the factor times the weight gives the number of micromoles of the compound. The number of micromoles of an element in a compound is the same as the number of the micromoles of the com-

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pound, or the number of microcoles times the number of atoms of a particular element in the compound.

It is hoped that these tables will be an aid to investigators. It is also entered as a plea for standardization of chemical units reported for culture media. It would be desirable that the molar unit, which has been used for decades in physiology, be adopted as the standard unit of concentration. Inasmuch as it is the number of reactive units and not the weight of these units which is important, molar concentrations are much more informative. For example, ratios of calcium/magnesium and nitrogen/phosphorus are frequently expressed in the literature on a weight basis. In this form, these data are not so meaningful as molar units, as it is the number of atoms rather than the weight of these elements which should be considered. By using molar concentrations, it is also possible to rapidly evaluate and prepare isotonic solutions and media.

There are certain instances though when it may be more advantageous to present concentrations on a mass basis rather than on a molar basis. For example, in comparing total dissolved material in natural waters, or in comparing experiments on the fertilization of lakes with culture media, the data are more appropriately handled on a mass basis. However, from a physiological standpoint, the advantages of the molar system are much more applicable.

TABLE 2 *Conversion factors for converting from milligrams of a micrcelement to milligrams of a compound, and from milligrams of a compound to micromoles of a compound*

	B	Co	Cu	Fe	Mn	Mo	Zn	Micromoles/milligram of compound
H_3BO_3	5.72							16.17
$CoCl_2 \cdot 6H_2O$		4.04						4.20
$CoSO_4 \cdot 7H_2O$		4.77						3.56
$CuCl_2 \cdot 2H_2O$			2.68					5.87
$CuSO_4 \cdot 5H_2O$			3.93					4.00
$FeCl_3 \cdot 6H_2O$				4.84				3.70
$Fe_2(SO_4)_3 \cdot 9H_2O$				10.06				1.78
$MnCl_2 \cdot 4H_2O$					3.60			5.05
$MnSO_4 \cdot 4H_2O$					4.06			4.48
$H_2MoO_4 \cdot H_2O$						1.88		5.56
$Na_2MoO_4 \cdot 2H_2O$						2.52		4.13
$ZnCl_2$							2.08	7.34
$ZnSO_4 \cdot 7H_2O$							4.40	3.48

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A Field Observation of Mortality of Marine Fish Larvae Due to Warming

Georges Bank, one of the most productive fishing grounds in the North Atlantic, provides a habitat for many populations of demersal fish, including cod, haddock, whiting, and several species of flounders. The non-tidal circulation pattern, being rotary, is favorable to maintaining these populations on the bank. However, there exists ample evidence that from time to time a considerable amount of Georges Bank water is carried off the southern part of the bank in the surface layer of the relatively warm slope water band (Bigelow 1929, Walford 1938). Although the mechanism of these movements is not fully understood, such a southerly drift could be caused by winds or by the absorption of coastal water by the large frictionally driven eddies that develop from time to time along the northern edge of the Gulf Stream (Iselin 1939). Iselin speculated that this warm slope water is unfavorable to large numbers of animals living near the edge of the continental shelf.

The North Atlantic Fishery Investigations of the U. S. Fish and Wildlife Service have conducted surveys of Georges Bank and the surrounding area in 1953, 1955, 1956, and 1957 in order to measure the relative abundance of fish eggs and larvae and to relate their distribution and mortality to hydrographic conditions. We have observed that a drift off the southeast edge of Georges Bank occurred in each of these years. Four 2-week cruises were made each year during February, March, April, and May. Operations included continuous Hardy Plankton Recorder hauls at the surface and at 10 meters, bathythermograph lowerings and surface salinity samples, drift bottle releases, and egg samples taken with a one-meter net for hatching and identification. The track of a typical cruise is shown in Figure 1. The drift of bottles, eggs, and larvae revealed that, in general, the water movement from the area of maximum spawning on Georges Bank was southerly.

One of the interesting features of the hydrography of the Georges Bank area is the band of relatively warm slope water along the southern edge of the bank. This slope water band is characterized by contrasting tongues of coastal and

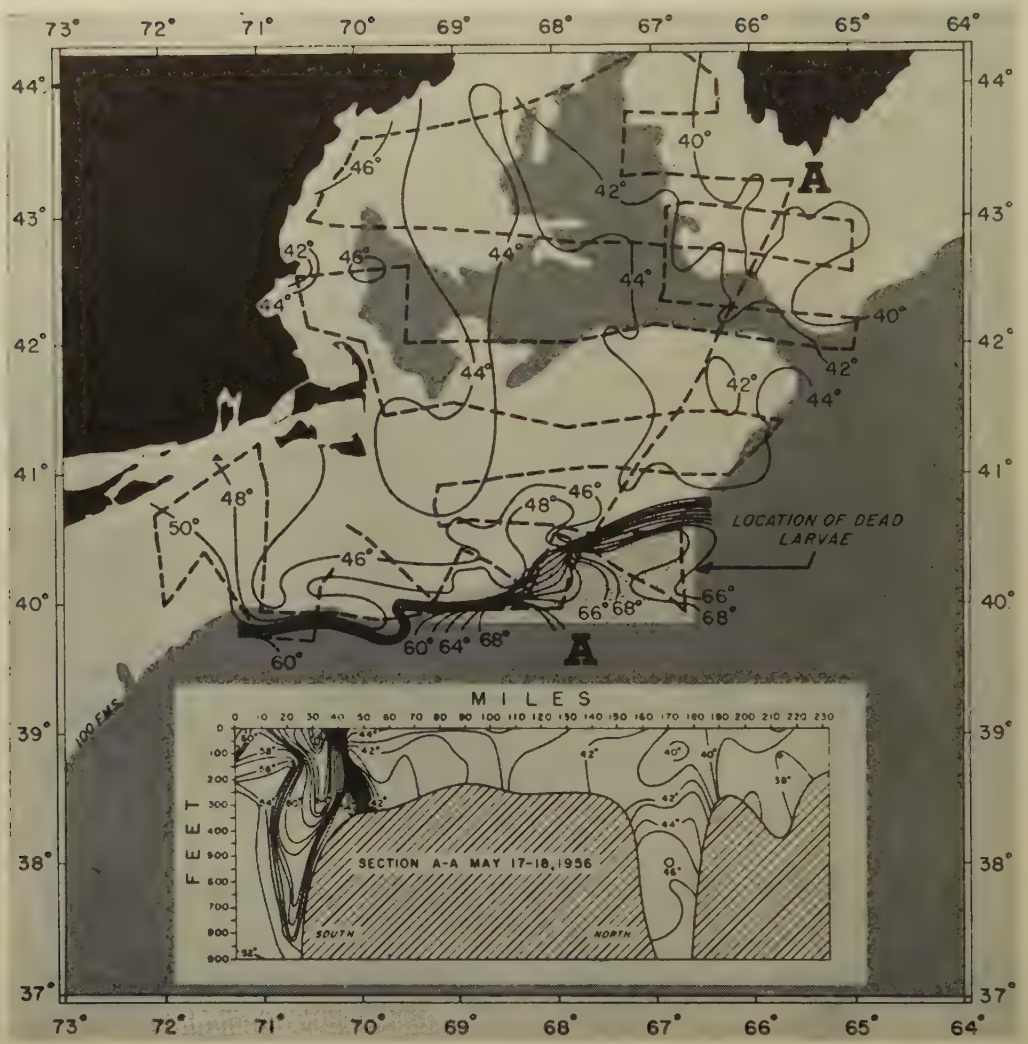

FIG. 1. Distribution of temperature and dead larvae, May 16–29, 1956.

Gulf Stream water. Here tropical plankton becomes mixed with coastal forms, and both drift slowly to the northeast, parallel to the Gulf Stream. In general, the northern boundary of this slope water band coincides with the 100-fathom contour line, but its position relative to Georges Bank, especially on the southeast part, varied during the 4 years of our surveys.

In May 1956 this slope water extended farther than usual over the southern edge of the bank (Fig. 1). Surface temperatures increased from 46°F to 68°F and surface salinities from 33‰ to 36‰ within a space of 10 miles. While we were cruising through this area, the temperature in our hatching jars (which are kept cool in a bath of running sea water) rose to 65°F, and most of the eggs died or aborted. No fish eggs, fish larvae, or invertebrates endemic to the Gulf of Maine-Georges Bank area were taken in the one-meter net surface hauls in this area, but juvenile stages of a number of tropical and oceanic species of fishes were caught, for example: mullett, *Mugil cephalus*; saurel, *Trachurus trachurus*; and lanternfish, *Myctophum humboltii*. The invertebrates in these samples were all tropical forms, the most numerous being the copepods, *Sapphirina gemma*, *Labidocera acutifrons*, *Undinula vulgaria*, and the euphausiid, *Euphausia krohnii*.

Later study of the Hardy Plankton Recorder gauzes revealed that certain sections contained many fish larvae in various stages of decomposition. These larvae were found in a particular area and only in the recorder towed at the 10-meter level. We first considered the possibility that there was a shortage of formalin in the storage tank of the recorder. However, only the

boreal forms were affected; all other plankters on the same sections were excellently preserved. This indicates that the affected larvae must have died some time before capture. The majority of these dead larvae were yellowtail flounder (*Limanda ferruginea*), but there were some whiting (*Merluccius bilinearis*) and a few larvae that appeared to be blennies.

We took the dead larvae only in the slope water (Fig. 1). Only boreal forms had died in this zone, while tropical and sub-tropical forms had not. The eggs and larvae of the species normally resident on Georges Bank, which were maintained in hatching jars, died during the time that the vessel was passing through the slope water. The only condition that changed during this time was temperature of the circulating sea water in which the hatching jars were immersed for cooling. The water inside the jars was the same as that in which the eggs had been originally collected, and thus only the temperature could have varied. These facts are all consistent with the conclusion that the eggs and larvae died as a consequence of temperature above their limit of tolerance.

Several observers have associated mass mortalities of adult fishes with abnormally low temperatures (Storey and Gudger 1936, Gunter 1941, Verrill 1901). Copenhagen (1935) described a periodic mortality of fish in the Walvis region associated with a marked increase in surface temperature. However, he concluded that death was due either to anoxia, hydrogen sulphide poisoning, or poisonous plankton. Wiborg (1948) noted the occurrence of large numbers of dead cod larvae in the Lofoten area in April 1930, but unfortunately no hydrographic observations were taken at the time. As far as we know, the present observations are the only ones that have been made which indicate a mortality of marine fish larvae as a result of warming.

Precise knowledge of the thermal requirements of the egg, developing embryo, and larval stages is meager.[1] A species of fish can exist only within a certain range of temperature. However, this range is more or less adjustable, the extreme limits being precisely set by conditioning (acclimation). In general, coldwater fishes have a narrower range of thermal tolerance than warmwater fishes. It appears also that marine species are more sensitive to temperature change than freshwater species, and that the thermal requirements of the very early stages are more exacting than that of the adult.

Carefully controlled laboratory experiments show that (1) the rate of temperature change is an important factor in mortalities, for it can outstrip the ability of the fish to acclimate to the change or move to a more favorable environment, (2) the upper lethal temperature is remarkably precise within species when repeated for fish from similar acclimation temperatures, and (3) the upper lethal temperature is closer to the acclimated temperature than is the lower lethal temperature.

Conditions on the southern edge of Georges Bank during May 1956 were such that Georges Bank larvae could be subjected to a sudden increase in temperature. The dead larvae averaged 2.5 mm in length, which approximates the hatching size of both yellowtail and whiting. Judging from the incubation rates of both yellowtail and whiting eggs and from the direction and rate of surface drift in the area, these larvae were probably hatched from eggs spawned approximately 25 miles north during the end of April. The temperature of the water mass in which the eggs and larvae drifted and to which they had been acclimated was approximately 44°F. The age of the larvae and their rate of drift indicate that they were subjected to a rise in temperature of over 20° in a period of probably less than 24 hours. At that planktonic stage of development, their swimming powers are much too feeble to enable them to move to a more favorable environment.

The above observations on flounder and whiting are very important, as the larvae of other and more important species such as haddock, cod, hake, and cusk may meet a similar fate. The extreme fluctuations in year-class strength of haddock, for instance, may be related to such a mortality in the larval stages. The drift of concentrations of haddock eggs and larvae has frequently been followed by us from the spawning centers on the northern edge to the southern edge of Georges Bank and then lost. For example, a large concentration of haddock larvae situated just north of the warmwater area in May 1956 had disappeared by June. It now appears that the reason we have not found larvae in the slope water zone before is that they had died in the warm water, and their bodies had disintegrated or sunk below the depth sampled.

This is further evidence to support the theory that larval fish swept off the southern edge of Georges Bank into the slope water zone are lost to the fishery.

[1] For a summary of work done on the thermal requirements of fishes, see Brett (1956).

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Preliminary Note on the Complete Larval Development of *Callinectes Sapidus* Rathbun under Laboratory Conditions[1]

A series of workers starting with Brooks (1882) and including Paulmier (1903), Hay (1904), Binford (1911), Churchill (1942), and Sandoz and Hopkins (1944) have attempted to rear *Callinectes sapidus* Rathbun through all larval stages in the laboratory but to date have been unsuccessful in raising them beyond the third zoeal stage. The information available on the later stages of development has, therefore, been obtained from reconstructions based on planktonic material. Churchill (1942) described his paper as filling the one remaining gap in our knowledge of the life history of the blue crab. Inasmuch as *Callinectes ornatus* and other species of the Portunidae are geographically sympatric with *C. sapidus* in much of its range and their life histories are unknown, we believe it is possible that the larvae of these species could easily be mistaken for one another. Hopkins (1944) is of the opinion that Churchill's description of the late zoeal stages (3–5) is in error and that positive identification of these stages would have to await rearing in the laboratory. We also feel that the only safe method of avoiding confusion with other species and obtaining accurate information concerning the larval development of *C. sapidus* would be to rear them from eggs in the laboratory. This method also has the advantage of giving more accurate information on the time of molting, the duration and mortality of each zoeal stage, and the time required for complete development to the crab.

Ovigerous *Callinectes sapidus* females were obtained from the Beaufort harbor, brought into the laboratory, and placed in battery jars containing running, filtered, sea water (23–27 p.p.t). The battery jars were tilted so that the overflow passed through a series of glass trays. When the eggs hatched the larvae were carried to the glass trays from which they were removed and segregated into mass cultures. They were then further subdivided into plastic compartmented boxes with one zoea per compartment. The zoeae were maintained at 23°–26°C and fed *Artemia* nauplii and fertilized *Arbacia* eggs. Some boxes were maintained on an Eberbach shaker (120/min), and others were stationary. The megalops and crab stages were fed the same diet plus beef liver. The compartments containing zoeae were observed daily for exuviae, and at this time the water and food were changed.

Hatching was observed, and the initial zoeae swam free of the egg mass without any intervening "prezoeal" stage. To confirm this some were removed immediately after hatching and examined under a microscope. They had dorsal and rostral spines, were positively phototrophic, and the maxillary hairs were not telescoped as has been described as characteristic of the "prezoea" by other workers. Near the end of the hatching period the female forcibly removed many of the remaining eggs with her pereiopods. These eggs were still surrounded by a membrane which enclosed the so-called "prezoea." None of these developed in the laboratory, although it is conceivable that they may in nature.

Duration of the individual zoeal stages varied from 3 to 8 days. Churchill (1942) described five zoeal stages in the larval development of *C. sapidus*. In this study, however, some larvae passed through a sixth, seventh, and even eighth zoeal stage. Successful metamorphosis to the megalops was observed only after seven zoeal stages. One eighth stage zoea metamorphosed to the megalops but died after one day. The overall time for zoeal development in the laboratory at 23°–26°C was 30 to 39 days.

The megalops stage persisted from 6 to 11 days and molted directly into the first crab stage. Thus the overall time from hatching to the first crab stage was 37 to 44 days.

The intermolt periods between the first 4 crab

[1] These studies were aided by a contract between the National Science Foundation and Duke University, G 4400.

stages were from 2 to 4 days. Mortality was highest during the first few days of zoeal development, at the time of the first molt, and at the molt from the last zoeal stage to the megalops. Survival in both the mass cultures and the segregated zoea was 1–8 per cent. Further studies are being made on the effects of salinity and temperature on the larval development.

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A Modified Flotation Technique for Sorting Bottom Fauna Samples[1]

The removal of organisms from benthic samples that contain a large quantity of organic debris is well known as a tedious and time-consuming task. The difficulty is compounded when particularly small invertebrates are numerous. A flotation procedure utilizing a sugar solution has proved efficient for sorting benthic samples collected from a variety of substrates.

One of the first investigators to propose the use of a high-density solution for separating invertebrates from debris in samples was Ladell (1936). He utilized a solution of magnesium sulfate to remove insects and other arthropods from soil samples. This technique was adopted by Beak (1938) who devised an apparatus for sorting bottom fauna from samples collected in streams. Lyman (1943) described a "salting-out" procedure in which he used a saturated solution of sodium chloride. Caveness and Jensen (1955) used a sugar solution for the separation of nematodes and their eggs from soils and plant tissues. Birkett (1957) used carbon tetrachloride for sorting living molluscs and invertebrates from debris consisting largely of shell.

The flotation principle has not been widely applied for sorting benthos samples except in situations where the substrate has been almost entirely inorganic. Moffett (1943) found the process practical for removing invertebrates from samples collected from a sandy, wave-swept shoal. Hunt (1953), in a study of the mayfly, *Hexagenia limbata*, reported that the "salting-out" procedure proved effective provided that residues were relatively heavier than the nymphs.

Specific gravity of organisms and debris.—Numerous tests have shown that the specific gravity of most organic debris (except fresh plant and algal material) in benthic samples is greater than 1.12. The specific gravity of most invertebrates is less than this value. When sample material is placed in a solution of this specific gravity, most of the invertebrates float to the surface and the bulk of organic material slowly sinks. This solution will float all organisms commonly encountered except insects with inorganic cases and molluscs.

Flotation time.—When using the flotation technique, the probability of recovering organisms is dependent, in part, on the length of time organisms remain at the surface. Animals shrink by fluid loss in any hypertonic solution and therefore increase in specific gravity. When the specific gravity of the invertebrates becomes greater than that of the solution, they sink unless retained by surface tension. The hypertonicity or osmotic pressure of different solutions at a given specific gravity is dependent on the molecular weight of the solute and the number of ions or molecules in solution. Therefore the flotation time should be longer in a sugar solution than in solutions of inorganic salts.

Tests were run to determine the difference in flotation time for various preserved organisms in solutions of calcium chloride (mol. wt. 111) and sucrose (mol. wt. 342). The specific gravity of each solution was 1.11. In order to prevent or-

[1] Contribution from the Michigan Institute for Fisheries Research. The assistance in the preparation of the manuscript by various members of the Institute staff including Dr. F. F. Hooper and Dr. P. H. Eschmeyer, and also Dr. K. F. Lagler of the Department of Fisheries, University of Michigan, is gratefully acknowledged.

ganisms from being retained by surface tension, a piece of clear plastic, bent to the shape of a U, was inverted into a finger bowl. In each test, the finger bowl was filled to a sufficient depth to submerge the plastic strip and 10 organisms of the group to be tested were then introduced into the solution underneath the plastic. The length of time that 50 per cent of the individuals remained floating up against the plastic was recorded as the flotation time. Tests were conducted with Tubificidae, the amphipod *Hyalella azteca*, larvae of the mayfly *Caenis* (2–3 mm long) and of the midges *Tanytarsus juncundus* (10–11 mm) and *Polypedilum* (3–4 mm).

TABLE 1. *Flotation time in minutes for organisms in solutions of calcium chloride and sucrose with a specific gravity of 1.11*

Organisms	Calcium chloride	Sucrose
Tubificidae	2	5
Hyalella azteca	4	9
Caenis sp.	20	30
Tanytarsus jucundus	17	45
Polypedilum sp.	23	90

TABLE 2. *Number of organisms removed from a bottom sample before and after preservation*

	Organisms			Time required (minutes)
	Tubificidae	Tendipedidae	Others	
Alive				
Flotation 1	1,145	31	2	15
Flotation 2	15	1	0	10
Total	1,160	32	2	25
Preserved				
Flotation 1	570	29	1	10
Flotation 2	269	3	1	8
Flotation 3	145	0	0	7
Hand-sort	158	0	0	45
Total	1,142	32	2	70

The length of time that the different organisms floated in the different solutions varied widely (Table 1). Tubificid worms are very susceptible to shrinkage and therefore had a short flotation time, whereas the midges floated for an appreciable period. Flotation time in most instances was more than twice as long in the sugar solution as in calcium chloride.

The original specific gravity of organisms can be approximately reconstituted by soaking in water. This was demonstrated with a group of 10 *Hyalella*. In this test the 10 organisms were alternately placed in a sugar solution until all of them had sunk and then in water for 10 minutes. The length of time that 50 per cent of these individuals floated in the sugar solution in three successive trials was 8, 6, and 6 minutes.

Flotation of fresh versus preserved samples.— The flotation time for living organisms is longer than for preserved specimens. The advisability of sorting samples while the organisms are alive or after preservation is dependent upon the type of organisms present in the sample. Certain insect larvae (Trichoptera, Ephemeroptera, Odonata, and others) cling to debris when alive, and do not float at the surface. As a result, they may not be recovered quantitatively. Therefore when these organisms are common, samples should be preserved before sorting by flotation. However, when aquatic oligochaetes occur in large numbers, it is desirable to sort the samples while the organisms are still alive. (The flotation time for this group when preserved was the

TABLE 3. *Number of organisms removed from bottom samples by flotation in a sugar solution and by hand-sorting*

	Organisms						Total organisms recovered	Time required (minutes)
	Hyalella azteca	Tendipedidae	Heleidae	*Caenis*	Trichoptera	Others		
Sample 1								
Flotation 1	270	93	14	24	19	8	428	20
Flotation 2	30	8	0	1	2	0	41	20
Total	300	101	14	25	21	8	469	40
Hand-sort	281	85	7	7	15	7	402	240
Sample 2								
Flotation 1	0	225	6	2	32	3	268	20
Flotation 2	1	28	6	0	3	1	39	20
Total	1	253	12	2	35	4	307	40
Hand-sort	1	176	5	0	7	7	196	300

shortest of all forms encountered.) These organisms remain alive for 10 to 15 minutes in the sugar solution, and this increases the flotation time to approximately 20 minutes.

In one test, a sample in which annelids were abundant was floated-out alive. The organisms were counted, then put back with the debris, and the sample was preserved and floated-out again. The sample was soaked in water for 20 minutes between each flotation period. During the first live flotation, approximately 99 per cent of the annelids present in the sample were recovered (Table 2). After the sample was preserved, only 85 per cent of the worms could be recovered in three flotation periods. The shorter flotation time after preservation made efficient recovery difficult.

Efficiency of flotation.—In order to compare the efficiency of hand-sorting and flotation, two preserved samples were first floated-out, the organisms, were counted, then put back with the debris. The samples were then sorted without the aid of flotation. In the two samples the total number of organisms recovered by hand-sorting amounted to 86 per cent and 64 per cent of the number removed during two flotation periods (Table 3). Each sample was sorted by flotation in less than one-fifth the time required for hand-sorting.

Flotation procedure adopted.—The following procedure has been adopted for sorting benthic samples. A sugar solution with a specific gravity of 1.12 is prepared (approximately 2.5 pounds of granulated sugar per gallon of solution). The specific gravity is determined with a hydrometer. The sample is drained as completely as possible through a sieve and placed in a 12- by 17-inch, white enamel dissecting pan. One-fourth of a quart to one quart of debris can be sorted at one time depending on the nature of the debris and the type and size of organisms in the sample. The material is flooded with 3 to 4 quarts of sugar solution and the debris is then stirred and distributed evenly over the bottom of the pan. Organisms are removed from the surface with a fine-mesh wire scoop. When no more organisms can be found, the sample is stirred and all additional organisms which come to the surface are removed. The sugar solution is then decanted off through the sieve and the sample is covered with water. While the sample is in water, the material is carefully examined for molluscs, insects in cases, and individuals entangled in vegetation. A careful examination will ordinarily take at least 20 minutes. After this period the water is poured off through the sieve, the sugar solution is again added, and all additional organisms are removed. If a large number of organisms are found during the second flotation period, the sample is soaked and floated-out again.

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A Note on Subterranean Nematodes from Chesapeake Bay, Md.

The subterranean water on marine beaches has been found to harbor a specialized and highly interesting fauna. The habitat was more or less discovered by Remane and Schulz (1934) and since then has been intensively studied by Remane and his co-workers, particularly by Gerlach (cf. Gerlach 1953), by Delamare-Deboutteville and co-workers (*e.g.* Delamare-Deboutteville 1954), and by some others (cf. Brinck *et al.* 1955). As a result the habitat is fairly well known along the German coast, parts of the Mediterranean coast, and parts of the West-European Atlantic coast. Other data have been published concerning such widely scattered localities as Madagascar, Southwest Africa, various points on the coasts of Brazil, Chile, Peru, and San Salvador. In North America the habitat has not been studied yet. Such an investigation, however, would be of interest, not least in order to test the thesis put forward re-

cently (Gerlach 1954, 1955) that the marine subterranean fauna is not only specialized, but also of world-wide distribution.

As a beginning a small scale investigation was undertaken of the subterranean fauna on Chesapeake Beach, Md.[1] The study focussed on the nematodes as the dominant group of the subterranean fauna (closely followed by the harpacticoid copepods) and also the best known one, largely due to Gerlach's work.

Collections were made on two points of Chesapeake Beach, *i.e.* a fine sand locality on South Beach and a coarse sand locality on North Beach, the two being about 1 km apart. Both beaches are flat. South Beach is protected by piers, but the sand is fairly clean. North Beach is unprotected and consequently more exposed to wave action. The salinity in this area of Chesapeake Bay according to Cowles (1930) varies between 9 and 13‰, according to Timm (1952) is 10.5‰. The sampling procedure was very simple. A hole was dug into the beach until water began to seep in from below. The water was scooped up and strained through a very fine piece of bolting silk. The material retained by the latter was examined under a dissecting microscope.

The specification of the samples taken is the following:

Sample No.	Distance from high water's edge (cm)	Depth (cm)	Date	Remarks
South Beach				
F-1	50	20	10/ 8/54	little detritus
F-2	50	20	10/21/54	in the vicinity black mud under
F-3	50	20	10/21/54	the surface
F-4	250	45	10/21/54	above a layer of black mud
North Beach				
C-1	50	20	10/ 8/54	little detritus
C-2	120	30	10/ 8/54	
C-3	500	50	10/ 8/54	sand somewhat finer, less shells
C-4	100	10	6/15/55	more detritus
C-5	300	30	6/15/55	

The mechanical analysis of the beach sand in the two localities yielded the following results (in % of the total weight of each sample):

Grain size (in μ)	South Beach	North Beach	
		Sample 1	Sample 2
>810	—	65.0	52.7
810–297	—	32.9	41.5
297–149	42.4	1.8	5.3
149–74	45.6	0.3	0.1
74–52	12.0	+	0.4

The accompanying fauna consisted mainly of copepods, turbellarians, collemboles, mites, and a few oligochaetes, polychaetes, ostracods, gastrotrichs, rotifers, and one tardigrade (*Batillipes mirus* or n.sp.).

The nematodes will be described separately (Wieser ms.). Their distribution is set out in Table 1.

As can be seen, both aspects of the subterranean habitat, the coarse sand and the fine sand, are characterized by a number of species. Whether this distinction holds for sands of similar composition on all beaches of Chesapeake Bay has to await further investigation.

The nematodes found may be divided into several ecological and geographical groups.

Ecological groups:

Species restricted to subterranean water and exposed parts of the beach: *Enoplus schulzi, Enoploides brunettii* var. *vectis, Enoplolaimus litoralis, Dolicholaimus benepapillosus, Anoplostoma exceptum, Hypodontolaimus schuurmansstekhoveni Theristus otoplanobius, Monhystera dahli.*

More eurytopic species, also occurring on mud or (though rarely) on algae: *Dorylaimus aestuarii, Oncholaimium oxure* var. *domesticum, Paracanthonchus caecus* (most eurytopic of all species), *Graphonema tentabunda, Ascolaimus elongatus, Odontophora axonolaimoides, Tripyloides gracilis, Theristus alternus, Theristus oistospiculum, Rhabditis marina.*

Both these ecological groups are about equally abundant in the coarse sand and in the fine sand localities.

Geographical groups:

Species restricted to Chesapeake Bay (cf. Timm 1952): *Odontophora axonolaimoides, Dorylaimus aestuarii*, and the five new species.

Species occurring on the east coast of both

[1] The investigation was carried out while the author held a fellowship of the International Cooperation Administration. The hospitality of Dr. G. Steiner, Nematology Division, Plant Industry Station, Beltsville, Md., in whose laboratory the material was worked up, is greatly appreciated.

TABLE 1. *Numerical distribution of nematodes in the subterranean water of two localities on Chesapeake Beach, Md.*

	South Beach				North Beach				
	F-1	F-2	F-3	F-4	C-1	C-2	C-3	C-4	C-5
Theristus otoplanobius Gerlach	9	12	1						
Tripyloides gracilis (Ditlevsen)	3	1	1						
Theristus alternus Wieser		5	3						
Metoncholaimus unguentarius n. sp.		7	2						
Odontophora axonolaimoides Timm	1		2						
Enoploides brunettii var. *vectis* Gerlach			1						
Dolicholaimus benepapillosus Schulz			1						
Hypodontolaimus schuurmans-stekhoveni Gerlach	7	15	37	3	1	2		6	
Dorylaimus aestuarii Timm		1	3		1				
Paracanthonchus caecus (Bastian)	5							5	3
Graphonema tentabunda (de Man)	1				1	1			
Rhabditis marina Bastian	1	1			2	1			
Enoplolaimus litoralis Schulz	1		1		3			24	40
Oncholaimium oxyure var. *domesticum* Chitwood			6		6			14	14
Theristus camelopardalis n. sp.					1	1		30	
Graphonema biserialis n. sp.					2	9	1		1
Theristus oistospiculum Allgén					6			1	
Anoplostoma exceptum Schulz					1			2	
Spilophorella simplex n. sp.						1	1		
Ascolaimus elongatus (Bütschli)								2	
Enoplus schulzi Gerlach						1			1
Oncholaimium priapulus n. sp.							1		
Monhystera dahli Wieser						7			

Americas: the above seven species plus *Enoploides brunettii* var. *vectis* and *Oncholaimium oxyure* var. *domesticum*.

Species occurring also in northern Europe (Baltic, North Sea): *Hypodontolaimus schuurmans-stekhoveni, Graphonema tentabunda, Theristus otoplanobius*.

Species occurring also in northern Europe and in the Mediterranean: *Dolicholaimus benepapillosus, Enoplolaimus litoralis, Anoplostoma exceptum, Ascolaimus elongatus*.

Species occurring on both sides of the Americas: *Theristus alternus*.

Species of probably worldwide distribution: *Enoplus schulzi, Paracanthonchus caecus, Tripyloides gracilis, Theristus oistospiculum, Rhabditis marina*.

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Townsend Cromwell, 1922–1958

The prominent oceanographer of the equatorial Pacific, Townsend Cromwell, was killed in an airplane crash on 2 June 1958. The accident, also fatal to B. Shimada, occurred near Guadalajara as these men were en route to join the Scot Expedition at Acapulco. Cromwell was Senior Scientist with the Inter-American Tropical Tuna Commission and Research Associate at Scripps Institution of Oceanography, La Jolla, California. His field of work was the physical environment and its relation to fisheries.

He became a weather officer in the Army Air Force during World War II. After receiving a B.A. degree from University of California (Los Angeles) in 1947, he returned to La Jolla, his boyhood home, as a student at Scripps, receiving an M.S. degree in oceanography from the University of California (La Jolla) in 1949. At Scripps he was strongly influenced by H. U. Sverdrup.

From 1949 to 1953 he was Oceanographer at Pacific Oceanic Fishery Investigations, Honolulu. O. E. Sette, then Director, and Cromwell initiated a far-sighted and intensive survey of the physical and biological characteristics of Pacific equatorial waters, which had been the subject of much speculation but little observation. For many months he participated in the field work from the HUGH M. SMITH, and for more and harder months he carried out the analysis of the observations. As a result of this pioneering work, the knowledge of the physical and biological structure of the equatorial Pacific Ocean has been vastly advanced. These achievements are the more remarkable when one notes that comparable surveys of the equatorial Atlantic and Indian oceans are still lacking.

Cromwell confirmed the existence of upwelling at the equator, disproved the existence of upwelling at the northern edge of the Equatorial Countercurrent, and originated a reasonable model of wind-induced current transport in the equatorial zone (*J. mar. Res.*, 1953). During these studies he recognized the significance of the unexpected drift of long-line fishing gear at the equator, and in 1952 he led a HUGH M. SMITH cruise using drogues in current measurements. Thus, he was responsible for the discovery of the Equatorial Undercurrent (*Science*, 1954), the fourth member of the equatorial current system (the North Equatorial Current, Equatorial Countercurrent, and South Equatorial Current having been known for a century). The existence of the Equatorial Undercurrent has been amply confirmed, during measurements completed a few days before Cromwell's death, by his colleagues J. A. Knauss and J. E. King (*Nature*, **182**, 1958). One result of four years in close collaboration with W. S. Wooster is a joint publication, in color, of a systematic analysis of data from the eastern tropical Pacific Ocean (*Bull. Scripps Inst. Oceanogr.*, 1958). The subject that aroused Cromwell's keenest interest is the structure and formation of oceanic discontinuities, both fronts and thermoclines (*Tellus*, 1956; *Bull. inter-Amer. trop. Tuna Comm.*, in press).

His work was characterized throughout by close collaboration with his associates, who became his devoted friends. His personal charm and modesty, together with his scientific interest, led a number of oceanographers to join his field of activity. These include E. D. Stroup, G. W. Groves, and myself.

Townsend Cromwell was born 3 November 1922 at Boston, Massachusetts, one of two sons of Richard and Lucile Cromwell. He married Katharine Huchthausen in 1947. Their children are Victoria, Katharine, Townsend, Carol Eugenia, and Elaine.

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Fellowships and Research Grants

1. The Division of Biological and Medical Sciences of the National Science Foundation announces that the next closing date for receipt of basic research proposals in the life sciences is May 15, 1959. Proposals received prior to that date will be reviewed at the summer meetings of the Foundation's advisory panels, and disposition will be made approximately four months following the closing date. Proposals received after the May 15, 1959, closing date will be reviewed following the fall closing date of September 15, 1959. Inquiries should be addressed to the National Science Foundation, Washington 25, D. C.

2. The Fellowship Office of the National Academy of Sciences—National Research Council has prepared "A Selected List of Major Fellowship Opportunities and Publications for Educational Support." This list includes fellowships at the undergraduate, pre-doctoral, and post-doctoral levels, available from various government agencies and private foundations. For further information contact the Fellowship Office, NAS-NRC, 2101 Constitution Avenue, Washington 25, D. C.

Conference on Waste Disposal

The University of California at Berkeley will host the "First International Conference on

Waste Disposal in the Marine Environment" during the summer of 1959. A tentative agenda of the three-day meeting includes (1) Waste disposal, (2) Public health, (3) Nearshore oceanography, (4) Receiving water analysis, (5) Marine biota, and (6) Estuarine hydrography. The program is being presented by the U. C. Sanitary Engineering Research Laboratory, the Institute of Marine Resources, and University Extension in cooperation with the California State Water Pollution Control Board. Further information may be obtained from the Department of Conferences, University Extension, University of California, Berkeley 4, Cal., USA.

Notice

There is a demand from libraries for complete sets of *Limnology and Oceanography* that cannot be met from the existing inventory. The American Society of Limnology and Oceanography is interested in purchasing complete volumes or separate numbers of Volumes I (1956) and II (1957). Direct inquiries to George H. Lauff, Secretary-Treasurer, ASLO, Department of Zoology. The University of Michigan, Ann Arbor, Michigan.

Correction

In my article entitled "An approach to some problems of secondary production in the western Lake Erie region" (*Limnology and Oceanography*, **3**(1): 15–28), an unfortunate error crept in on lines 7, 9, and 15 in the left-hand column on page 18. In four places, mg was written, whereas μg was intended.

In addition, as pointed out in American Public Health Association, "Standard methods for the examination of water and sewage" (9th ed., p. 20), "Loss on ignition is a relative, not a precise determination." For this reason, the values reported in my paper for dissolved organic matter (pp. 19–20, and Table 3) are subject to this limitation.

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BOOK REVIEWS

BEVERTON, RAYMOND J. H., AND SIDNEY J. HOLT. 1957. **On the Dynamics of Exploited Fish Populations.** Gt. Britain, Fishery Invest., Ser. II, Vol. XIX. 533 pp. 126/— (= U. S. $17.64).

It is brash to attempt to review in short compass a 533-page book which the authors have themselves required 8 pages to summarize. I have met both authors, and Sidney Holt was our biometrics instructor in a six-weeks FAO course which I conducted in Istanbul in 1953. They have done a truly stupendous and painstaking job in producing a monograph on population dynamics which will enhance the meaning of fishery dynamics for a long period. It should prove equally useful to biometricians studying other organisms.

Any comments which I make in the invidious role of a critic are not meant to detract from the value of a work that belongs on the shelf of any serious student of the subject. For the average biologist the very multiplicity of formulae—the count is 419 formally numbered formulae plus divers minor formulae—is too formidable to cope with. The number of formulae is further complicated by radical departure from the simplicity of R. A. Fisher. The authors list 120 different symbols, 12 of which are used with two meanings and 2 with three meanings. The extensive references throughout the text to previous sections of the book makes reading laborious.

The classical approach in fisheries has been from the actual to the theoretical. The authors have reversed this at times using a highly theoretical and purely mathematical approach. It is too soon to form a competent judgment of the results. For demersal fisheries the authors have been able in many instances to find data which support their formulae. It must here be pointed out, however, that some of the assumptions which apply to these relatively simple cases may often fail when applied to pelagic or anadromous species.

The chief shortcoming of the theoretical approach is the necessity for always making a number of assumptions. Only rarely does one find all of these assumptions satisfied in any set of actual field data. Granted that theory may sometimes enable revision of data collecting methods so as to satisfy some assumption, yet the biologist must make decisions now based on the data available.

The case which the authors attempt to make for "permanent self-induced oscillations in population abundance" (p. 57) for populations with a dome-shaped reproductive curve having a steep right limb, while possibly mathematically sound, must be based on a stable environment. Since such an environment is practically non-existent the correctness of this theory must await corroboration.

The authors were evidently unaware of the occasional deaths of millions of marine fish in the Laguna Madre of Texas reported by Gunter, Hedgpeth and others (footnote on p. 68), from excessively high salinities.

In developing the formulae for fishing mortality (pp. 89–91) the assumption is made that by taking a small enough unit of time the natural mortality occurring simultaneously can be ignored. This could as well be stated in reverse.

Owing undoubtedly to the authors familiarity with year-round demersal fisheries the sections on gear saturation and seasonal variation in fishing (pp. 94–95) are disappointingly brief and offer no adequate solutions for analyzing the types of data usually available for determining abundance in anadromous and many pelagic fisheries.

In developing methods for standardizing fishing effort the authors claim a linear fit between gross tonnage and the power factor of trawlers; yet the linear curves shown (p. 175) should obviously be curvilinear. The reverse is true of the length versus power factor curve on p. 176. This effort to use only proportional linear curves is understandable since it permits easier use of the British trawl statistics which give hours of trawling per gross ton-hour. However, there is little mention of age of vessels, an important factor of gear efficiency in many fisheries.

In selecting examples to illustrate the derivation of the natural mortality coefficient the authors chose data from the Fraser River sockeye fisheries [Rounsefell. 1949. Biometrics, **5**(2)]. Unfortunately, the coefficient which they ascribe to "residual fishing during the period as well as true natural mortality" does not contain natural mortality. The escapement of salmon in the example was derived from the gillnet catches assuming the absence of natural mortality. The very high coefficient they show is due entirely to fishing by other gears.

In discussing the relation between potential egg production and recruitment the authors have used throughout the text a curve approaching an upper asymptote. They cite a few examples to show that this type of reproductive curve not only applies to marine fishes but also applies to salmon. They thus dismiss the theoretical interpretation of a dome-shaped reproductive curve for salmon as postulated by Ricker [1954. Stock and Recruitment. J. Fish. Res. Bd. Canada, **11**(5)]. In the only example for salmon containing over 7 years of data the authors used the Fraser River sockeye, the runs of which are composed of several distinct races that merely use the river as a means of ingress and egress from their individual lake systems. Full confirmation of Ricker's hypothesis has since been shown using 59 years of data for the Karluk River sockeye [Rounsefell. 1958. Bull. U. S. Fish and Wildlife Service, **58**(130)].

The use of multitudinous symbols with tiny subscripts makes use of a reading lens almost necessary. No distinction is clearly made between formulae that have been corroborrated by adequate field data and those which are purely theoretical concepts. Perhaps the most noticeable omission is the failure to include tests of significance.

In summary, this book is a veritable gold mine of information for biometricians interested in population dynamics. Although the reviewer may disagree on a few points, this in nowise detracts from the overall value of this exhaustive treatise.

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MOORE, HILARY B. 1958. **Marine Ecology.** John Wiley & Sons, Inc., New York. 493 pp. $9.50.

Oceanography embraces study of all aspects of the sea. Marine ecology, the science of interrelations between living marine organisms and their environment, draws upon several branches of oceanography (for environmental factors) and in addition includes study of individuals and species (autecology) and—the unique aspect of the science—of groups of marine organisms associated together as populations, communities, and ecosystems (synecology). The magnitude of the task of synthesis of knowledge in marine ecology in the form of a text for student use is indicated by the substantial three dimensional geographic domain encompassed by, the large number and complexity of interrelated functional units in, and the incompleteness of knowledge in many areas of the field.

Such a text has been attempted by Hilary B. Moore in *Marine Ecology*. The author expresses his concept of ecology and describes the content of his book as follows (p. 16): "A survey of the present status of ecological knowledge cannot be made to fit into a concise and orderly pattern and would, in fact, suffer if so limited. There is too much interconnection of effects. In succeeding chapters, the various ecological factors are considered in turn, and examples are given of the ways in which they have been found to affect different organisms. After this an account is given of the more important types of environment, together with an outline of the variations and significance of the various factors in each. These environments range from the comparatively stable and simple abyssal regions to the highly complex intertidal zone. Finally, these same environments are considered in terms of the organisms inhabiting them with a discussion of the present status of knowledge of typical forms."

The book possesses an attractive format and is enhanced by 214 illustrations and 71 tables. It contains 26 pages of references, principally in English (the choice was deliberate to meet needs of English-speaking students), which represent a wide search of the literature. The text introduces a classification of plants and animals designed to facilitate the placing of unfamiliar genera in appropriate taxa. This is helpful since the author draws examples from marine areas over many different parts of the earth.

The volume strongly stresses environmental ecology, autecology, and habitat ecology Eighty-two pages provide good coverage of physical and chemical environmental factors. The next 24 pages are devoted to "biological environmental factors" and cover the topics: food, crowding, dispersal, predation, and interaction of multiple factors. The subject of "dispersal," however, does not fit smoothly in a framework of "biological environmental factors." A description of major habitats occupies the following 81 pages. Penetration of light in the upper oceanic zone is well treated, but discussion of the physical characteristics of estuaries conspicuously omits such aspects of circulation as non-tidal flow and the significance of this to planktonic larvae of estuarine organisms. A lengthy explanation (pp. 195–8) on the "effect of . . . salinity distribution on the variations in optimum levels of various types of organisms in an estuary" is not clear. Two pages of discussion of pollution (the only treatment of the subject in the text) are appended to the section on salinity in the estuary; since pollution is not restricted to estuaries, it might better be treated in a separate section. In view of the significance of salt marshes and mangroves, it is unfortunate that so little space is devoted to their habitats. The next 204 pages cover organisms by habitats. The best sections are those on organisms of the upper oceanic zone, coral reefs, and intertidal rocky shores. That on organisms of intertidal rocky shores is disproportionately long, which probably reflects accessibility and more active study of this marine area. The section on inshore pelagic organisms devotes 22 pages to the ecology (much of it more conservation than ecology) of commercial inshore fin fisheries; by contrast the section on sublittoral "bottom communities" reviews briefly principally Petersen's bottom community studies and entirely omits review of and reference to the substantial ecological literature on the various species of commercial oysters. Likewise no consideration is given to dominant forms like *Callinectes* which are equally active on the bottom and in the water. Combination of the sections on major habitats and organisms would have eliminated some duplication.

The book closes with a chapter of 8 pages entitled "Review." The author points out here (as well as in the "Introduction") that ". . . in the whole field of ecology, there are very few

general laws or principles that have been enunciated." One wonders, then, of the effectiveness of the labors of W. C. Allee, A. E. Emerson, O. Park, T. Park, and K. P. Schmidt in their monumental *Principles of Animal Ecology* (1949), of G. L. Clarke in his *Elements of Ecology* (1954), and of E. P. Odum and H. T. Odum in *Fundamentals of Ecology* (1959)—just to mention a few of the contributions on the synthesis of ecological principles. The author states that of the principles which are generally accepted, some are of only limited application and others do not apply to marine forms. In this connection this reviewer would like to point out that no aspect of marine ecology has been investigated exhaustively enough to demonstrate whether ecological principles enunciated in connection with terrestrial or fresh water do not apply. The author also suggests (p. 419) that the community (discussion of which is omitted throughout most of the text) "may be something more than the sum of its constituents," since "factors controlling the community differ from those controlling individual species" (?); and that "when the autecologist knows the characteristics of all the possible replacement units" (alternative species or physiological races which may be substituted to meet changing conditions) "in a given community, then he will know all the possible manifestations of that community." This possibility appears incongruous with the concept expressed by many ecologists that a biotic community has characteristics additional to its individual and population components.

According to the author ecology during its evolution has tended to concentrate on two approaches—autecology (where the species is the unit) and synecology (where the community is the unit). He suggests that "a rift has developed between the two schools of thought, and the protagonists of one sometimes fail to appreciate the true value of the other" (p. 7). He recommends that a third approach (which he terms "integral ecology") recombine autecology and synecology into the original whole. Actually, however, there are no sharp boundaries between autecology and synecology: these concepts simply represent two ways of approaching ecological problems. The book suffers occasionally from inadequate definition of terms introduced by the author and lack of integration of these with ecological terms in more common usage in the literature. For example, one wonders if the author's use of "integral ecology" is synonymous with the current use of "ecosystem"?

It is regrettable that the text gives evidence of inadequate editing and hasty preparation. A superficial count disclosed some 15 conspicuous typographical errors. Four of these concerned misspelling of authors' names. A reference to Tressler (1951) is cited in the text (p. 362) but no such reference is listed under the section on "References"; likewise the year of publication to a reference by Orr and Moorhouse (p. 52) is omitted. On page one it is stated that a *Treatise on Marine Ecology and Palaoecology* will be published, and later (p. vi) that the *Treatise* has been published. The author uses the terms "tropism" and "taxis" interchangeably, yet refers to Fraenkel and Gunn *The Orientation of Animals* (1940) in which tropism is used to refer to orientation of sessile organisms, and taxis to that of motile organisms. The eastern American oyster is now in the genus *Crassostrea* rather than *Ostrea* (p. 28). The contribution to marine ecology of the effect of temperature on grasshopper eggs (p. 32) is questionable. On p. 245 the statement is made that nannoplankton have proved to be an ideal food for such species as oyster larvae and *Calanus*; recent researches, however, show at least for the oyster that only certain species provide good food. On p. 117 the author notes (without citation) that the shape of the hole bored by carnivorous gastropods is frequently characteristic of a particular species of gastropod (to my knowledge available research is insufficient to permit his generalization). No reference is given to the interesting possibility (p. 309) that "clam beds in North America (*Venus mercenaria*), which have declined in value through lack of settlement of spat, may often be recolonized when soil is spread over the surface . . . " (this refers to investigations of *Mya*, not *Venus*); or that "food material contained in the larvae which disappear by predation is not wholly lost to the parent species, since many of the parents have been recorded as feeding to a considerable extent on their own young when these are ready to settle" (p. 315). And on another page (p. 375–6) the author states that "Boring of the *Mytilus* shells, by *Purpura*, is performed entirely by radula action, without the assistance of any acid secretion." (It would be interesting to see the evidence for this in view of research appearing during the last two decades on boring mechanisms in muricid and naticid gastropods).

The reviewer found the book difficult reading, principally because more care was not exercised by the author in phrasing sentences; for example: "It is also known that many of the species composing this deep layer, in the region where it has been demonstrated, are very wide distribution elsewhere" (p. 223); and "Marshall and Orr (1931) found a surprising ability to remove sediment from the surface of the coral" (p. 329).

This volume, although entitled "Marine Ecology," makes only passing mention of, or omits entirely, such significant areas of marine ecology as species population, interspecies populations, communities, and ecosystems. It is difficult to agree with the author (p. 412) that "in the present state of our knowledge, attempts to regiment ecological observations into a set pattern have too often resulted in obscuring vitally important points"; and it is not out of place to suggest that he has, in fact, regimented

the results of his many years of research and his wide coverage of the marine ecological literature into the specific patterns which he formulated for his book. In the review, study, and synthesis leading to the preparation of his book, the author placed himself in an unusually favorable position to attempt application of the principles of general ecology to marine ecology and to formulate new concepts; it is disappointing that more of this was not done.

Although prepared by the author for student use, this text does not lend itself to use in introductory courses in marine ecology, principally because of the imbalance of the subject matter of the book, the presence in it of numerous typographical errors, and evidence of hasty preparation. It should, however, prove useful to investigators in the field of marine ecology as a reference volume. In view of accelerated research and increased course offerings in marine ecology, there is an urgent need for a well balanced textbook in this branch of oceanography written for upper undergraduate students at the college and university level. Perhaps Professor Moore would be willing to revise his book to fill this need.

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